12 December 2024
EMA/1684/2025
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Zefylti

International non-proprietary name: filgrastim

Procedure No. EMEA/H/C/006400/0000

Note

Assessment report as adopted by the CHMP with all information of a commercially confidential nature deleted.

Official address Domenico Scarlattilaan 6 ● 1083 HS Amsterdam ● The Netherlands
Address for visits and deliveries Refer to www.ema.europa.eu/how-to-find-us
Send us a question Go to www.ema.europa.eu/contact **Telephone** +31 (0)88 781 6000 An agency of the European Union

Table of contents

List of abbreviations

ADA	anti-drug antibodies
ADE	adverse drug experience
AE	adverse event
AEX	anion exchange chromatography
ALP	alkaline phosphatase
ALT	alanine aminotransferase
AML	acute myeloid leukaemia
ANC	absolute neutrophil count
ANOVA	analysis of variance
ARDS	acute respiratory distress syndrome
AST	aspartate aminotransaminase
AUC	area under the concentration-time curve
AUC(0-inf)	AUC from time zero extrapolated to infinity
AUC(0-t)	AUC up to the last measurable concentration
AUEC	area under the effect curve
AUEC(0-t)	AUEC from time 0 to the time of the last quantifiable concentration
BET	bacterial endotoxin
BLQ	below the lower limit of quantification
BM	bone marrow
BMI	body mass index
CD	cluster of differentiation
CEX	cation exchange chromatography
CI	critical intermediate
CI	confidence interval
CIN	chemotherapy-induced neutropenia
Cmax	maximum observed concentration
CoA	certificate of analysis
CPP	critical process parameter
CQA	critical quality attributes
CRO	contract research organisation
CTCAE	common terminology criteria for adverse events
Ctrough	pre-dose concentration
CV	coefficient of variation
DC	dendritic cell
DNA	deoxyribonucleic acid
DS	drug substance
DSC	differential scanning calorimetry
DSN	duration of severe neutropenia
EAC	equivalence acceptance criteria
ECG	electrocardiogram
eCRF	electronic case report form
EDC	electronic data capture
ELISA	enzyme-linked immunosorbent assay
EMA	European Medicines Agency
Emax	maximum effect
EPCB	end of production cell bank
ESI LC-MS	electrospray ionisation liquid chromatography tandem mass spectrometry
EU	European Union
FMEA	failure modes and effects analysis
FN	febrile neutropenia
FTIR	Fourier transform infrared spectroscopy
GC	gas chromatography
GCP	good clinical practice
G-CSF	granulocyte-colony stimulating factor
G-CSFR	granulocyte-colony stimulating factor receptor
GMP	good manufacturing practice
GMR	geometric mean ratio
GvHD	graft-versus-host disease
HBsAg	hepatitis B surface antigen
HCD	host cell DNA
HCP	host cell protein

HCT	hematopoietic cell transplantation
HCV	hepatitis C virus
HDPE	high density polyethylene
HIV	human immunodeficiency virus
HMW	high molecular weight
HMWs	high molecular weight species
HPLC	high performance liquid chromatography
HSA	human serum albumin
IB	inclusion body
IB	investigator's brochure
ICF	informed consent form
ICH	International Conference on Harmonization
icIEF	imaged capillary iso-electric focusing
ICSR	individual case safety report
IEC	independent ethics committee
IFN-γ	interferon gamma
IL	interleukin
IMP	investigational medicinal product
INN	international non-proprietary name
IPC	in-process control
IPT	in-process test
IPTG	isopropyl ß- d-1-thiogalactopyranoside
IRB	institutional review board
IRS	internal reference standard
ISO	International Organization for Standardization
ISR	injection site reaction
IV	intravenous
KD	equilibrium dissociation constant
kDa	kilodalton
KPP	key process parameter
LC	liquid chromatography
LC-MS	liquid chromatography mass spectrometry
LLOQ	lower limit of quantification
LOD	limit of detection
LOQ	limit of quantitation
MAA	marketing authorisation application
MCB	master cell bank
MCP-1	monocyte chemoattractant protein -1
MedDRA	medical dictionary for regulatory activities
MFI	micro-flow imaging
mM	millimolar
MS	mass spectrometry
MU	million units
NA	not applicable
NAb	neutralising antibody
NBOp	notified body opinion
NCPP	non-critical process parameter
NFgB	nuclear factor kappa B
NIBSC	National Institute for Biological Standards and Control
NMR	nuclear magnetic resonance spectroscopy
NOR	normal operating range
NRBC	nucleated red blood cell
NSG	needle safety guard
OD	optical density
PAMP	pathogen associated molecular patterns
PAR	proven acceptable range
PBMC	peripheral blood mononuclear cells
PBPCs	peripheral blood progenitor cells
PC	process characterisation
PCB	primary cell bank
pCPP	potential critical process parameters
PD	pharmacodynamics
PETG	polyethylene terephthalate glycol
PFS	prefilled syringe

pI	isoelectric point
PK	pharmacokinetic
PPQ	process performance qualification
PRS	primary reference standard
PS80	polysorbate 80
PSUR	periodic safety update report
PT	preferred term
PVC	polyvinyl chloride
qPCR	quantitative PCR
RANTES	regulated on activation, normal T-cell expressed and secreted protein
RBA	relative binding affinity
rG-CSF	recombinant granulocyte-colony stimulating factor
RMP	reference medicinal product
RMP	risk management plan
RP-HPLC	reverse phase high performance liquid chromatography
RP-UPLC	reverse phase ultra performance liquid chromatography
SAE	serious adverse event
SAP	statistical analysis plan
SAS	statistical analysis software
SC	subcutaneous
SCN	severe congenital neutropenia
SD	standard deviation
SDS-PAGE	sodium dodecyl sulphate polyacrylamide gel electrophoresis
SEC	size exclusion chromatography
SEC-MALS	size exclusion chromatography with multi-angle static light scattering
SE-HPLC	size exclusion-high performance liquid chromatography
SmPC	summary of product characteristics
SN	severe neutropenia
SoA	schedule of activities
SOC	system organ class
SOP	standard operating procedure
SPR	surface plasmon resonance
SRS	secondary reference standard
SS	system suitability
STAT3	signal transducer and activator of transcription 3
SV-AUC	sedimentation velocity-analytical ultra-centrifugation
T½	terminal elimination half-life
TEAE	treatment-emergent adverse event
TLR	toll-like receptor
Tm	thermal transition midpoint
Tmax	time of maximum concentration
TMB	tetramethylbenzidine
TNFα	tumour necrosis factor alpha
TSE	transmissible spongiform encephalopathy
UF/DF	ultrafiltration/diafiltration
UK	United Kingdom
ULN	upper limit of normal
US	United States
USP	U.S. Pharmacopoeia
UV	ultraviolet
UV CD	ultraviolet circular dichroism spectroscopy
UV-VIS	ultraviolet - visible
WBC	white blood cell
WCB	working cell bank
WCC	white cell count
WFI	water for injection

1. Background information on the procedure

1.1. Submission of the dossier

The applicant CuraTeQ Biologics s.r.o. submitted on 10 November 2023 an application for marketing authorisation to the European Medicines Agency (EMA) for Zefylti, through the centralised procedure falling within the Article 3(1) and point 1 of Annex of Regulation (EC) No 726/2004.

The applicant applied for the following indication:

Zefylti is indicated for the reduction in the duration of neutropenia and the incidence of febrile neutropenia in patients treated with established cytotoxic chemotherapy for malignancy (with the exception of chronic myeloid leukaemia and myelodysplastic syndromes) and for the reduction in the duration of neutropenia in patients undergoing myeloablative therapy followed by bone marrow transplantation considered to be at increased risk of prolonged severe neutropenia. The safety and efficacy of Zefylti™ are similar in adults and children receiving cytotoxic chemotherapy.

Zefylti is indicated for the mobilisation of peripheral blood progenitor cells (PBPCs).

In patients, children or adults, with severe congenital, cyclic, or idiopathic neutropenia with an ANC (Absolute Neutrophil Count) of ≤ 0.5 x 10^9/l, and a history of severe or recurrent infections, long term administration of Zefylti is indicated to increase neutrophil counts and to reduce the incidence and duration of infection-related events.

Zefylti is indicated for the treatment of persistent neutropenia (ANC less than or equal to 1.0 x 10^9/L) in patients with advanced HIV infection, in order to reduce the risk of bacterial infections when other options to manage neutropenia are inappropriate.

1.2. Legal basis, dossier content

The legal basis for this application refers to:

Article 10(4) of Directive 2001/83/EC – relating to applications for biosimilar medicinal products.

The application submitted is composed of administrative information, complete quality data, appropriate non-clinical and clinical data for a similar biological medicinal product.

The chosen reference product is:

Medicinal product which is or has been authorised in accordance with Union provisions in force for not less than 10 years in the EEA:

- Product name, strength, pharmaceutical form: Neupogen, 30 and 48 MU, solution for injection in pre-filled syringe / concentrate for solution for infusion
- Marketing authorisation holder: Amgen Europe B.V.
- Date of authorisation: 17-07-2001
- Marketing authorisation granted by:
  - Member State (EEA): The Netherlands
    - MRP
- Marketing authorisation number: RVG 26386 and RVG 26387

Medicinal product authorised in the Union/Members State where the application is made or European reference medicinal product:

- Product name, strength, pharmaceutical form: Neupogen, 30 and 48 MU, solution for injection in pre-filled syringe / concentrate for solution for infusion
- Marketing authorisation holder: Amgen Europe B.V.
- Date of authorisation: 17-07-2001
- Marketing authorisation granted by:
  - Member State (EEA): The Netherlands
    - MRP
- Marketing authorisation number: RVG 26386 and RVG 26387

Medicinal product which is or has been authorised in accordance with Union provisions in force and to which bioequivalence has been demonstrated by appropriate bioavailability studies:

- Product name, strength, pharmaceutical form: Neupogen, 30 and 48 MU, solution for injection in pre-filled syringe / concentrate for solution for infusion
- Marketing authorisation holder: Amgen Europe B.V.
- Date of authorisation: 17-07-2001
- Marketing authorisation granted by:
  - Member State (EEA): The Netherlands
    - MRP
  - (Union) Marketing authorisation number(s): RVG 26386 and RVG 26387
- Bioavailability study number: BP13-101

1.3. Information on paediatric requirements

Not applicable.

1.4. Information relating to orphan market exclusivity

1.4.1. Similarity

Pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, the applicant did not submit a critical report addressing the possible similarity with authorised orphan medicinal products because there is no authorised orphan medicinal product for a condition related to the proposed indication.

1.5. Scientific advice

The applicant did not seek scientific advice from the CHMP.

1.6. Steps taken for the assessment of the product

The Rapporteur and Co-Rapporteur appointed by the CHMP were:

Rapporteur: Outi Mäki-Ikola Co-Rapporteur: Daniela Philadelphy

The application was received by the EMA on	10 November 2023
The procedure started on	28 December 2023
The CHMP Rapporteur's first assessment report was circulated to all CHMP and PRAC members on	15 March 2024
The CHMP Co-Rapporteur's first assessment report was circulated to all CHMP and PRAC members on	28 March 2024
The PRAC Rapporteur's first assessment report was circulated to all PRAC and CHMP members on	28 March 2024
The CHMP agreed on the consolidated list of questions to be sent to the applicant during the meeting on	25 April 2024
The applicant submitted the responses to the CHMP consolidated list of questions on	22 July 2024
The CHMP Rapporteurs circulated the CHMP and PRAC Rapporteurs Joint assessment report on the responses to the list of questions to all CHMP and PRAC members on	22 August 2024
The PRAC agreed on the PRAC assessment overview and advice to CHMP during the meeting on	5 September 2024
The CHMP Rapporteurs circulated the updated CHMP and PRAC Rapporteurs joint assessment report on the responses to the list of questions to all CHMP and PRAC members on	12 September 2024
The CHMP agreed on a list of outstanding issues in writing to be sent to the applicant on	19 September 2024
The following GMP inspection was requested by the CHMP and their outcome taken into consideration as part of the quality/safety/efficacy assessment of the product:	
– A GMP inspection at one manufacturing site in India was conducted between 8 April 2024 – 12 April 2024. The outcome of the inspection carried out was issued on	12 November 2024
The CHMP Rapporteurs circulated the CHMP and PRAC Rapporteurs joint assessment report on the responses to the list of outstanding issues to all CHMP and PRAC members on	26 November 2024
The CHMP Rapporteurs circulated the updated CHMP and PRAC Rapporteurs joint assessment report on the responses to the list of outstanding issues to all CHMP and PRAC members on	05 December 2024
The CHMP, in light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a marketing authorisation to Zefylti on	12 December 2024

2. Scientific discussion

2.1. About the product

The active substance of Zefylti (also referred as BP13) is filgrastim (ATC code: L03AA02). Filgrastim is a human granulocyte colony-stimulating factor (G-CSF) produced by recombinant DNA technology. Endogenous G-CSF is a lineage specific colony-stimulating factor which is produced predominantly by monocytes-macrophages, fibroblasts, and endothelial cells. G-CSF regulates the production of neutrophils within the bone marrow (BM) and affects neutrophil progenitor proliferation, differentiation, and selected end-cell functional activation.

BP13 is proposed as a biosimilar of the EU-approved Neupogen.

The proposed indications for BP13 are identical to the EU-approved indications of Neupogen:

Zefylti is indicated for the reduction in the duration of neutropenia and the incidence of febrile neutropenia in patients treated with established cytotoxic chemotherapy for malignancy (with the exception of chronic myeloid leukaemia and myelodysplastic syndromes) and for the reduction in the duration of neutropenia in patients undergoing myeloablative therapy followed by bone marrow transplantation considered to be at increased risk of prolonged severe neutropenia. The safety and efficacy of Zefylti are similar in adults and children receiving cytotoxic chemotherapy.

Zefylti is indicated for the mobilisation of peripheral blood progenitor cells (PBPCs).

In patients, children or adults, with severe congenital, cyclic, or idiopathic neutropenia with an absolute neutrophil count (ANC) of ≤0.5 x 10^9/L, and a history of severe or recurrent infections, long term administration of Zefylti is indicated to increase neutrophil counts and to reduce the incidence and duration of infection-related events.

Zefylti is indicated for the treatment of persistent neutropenia (ANC less than or equal to 1.0 x 10^9/L) in patients with advanced HIV infection, in order to reduce the risk of bacterial infections when other options to manage neutropenia are inappropriate.

The proposed recommended dose and route of administration of BP13 are also the same as for Neupogen.

2.2. Type of application and aspects on development

The marketing authorisation application (MAA) for Zefylti was developed as a proposed biosimilar of Neupogen (filgrastim) in line with Article 10(4) of Directive 2001/83/EC. The clinical PK/PD study BP13-101 was conducted using as reference product Neupogen (Amgen Europe BV), authorised within EU via mutual recognition pathway.

The proposed indication for Zefylti is identical to the EU-approved indication of Neupogen.

The following guidelines were taken into consideration in the development of Zefylti:

- Draft "Guideline on Similar Biological Medicinal Products containing Biotechnology-Derived Proteins as Active Substance: Non-Clinical and Clinical Issues (EMEA/CHMP/BMWP/42832/2005 Rev 1)"
- "Guideline on Similar Biological Medicinal Products (CHMP/437/04 Rev 1)"
- Draft EMA "Guideline on similar biological medicinal products containing recombinant granulocyte-colony stimulating factor (rG-CSF) (EMEA/CHMP/BMWP/31329/2005 Rev 1)"

- The current "Guideline on similar biological medicinal products containing recombinant granulocyte-colony stimulating factor (rG-CSF) (EMEA/CHMP/BMWP/31329/2005)"

According to the draft guideline "Guideline on similar biological medicinal products containing recombinant granulocyte-colony stimulating factor (rG-CSF) (EMEA/CHMP/BMWP/31329/2005 Rev 1)" pivotal evidence for similar efficacy can be derived from the similarity demonstrated in physicochemical, functional, PK and PD comparisons, and therefore a dedicated comparative efficacy trial is not considered necessary.

2.3. Quality aspects

2.3.1. Introduction

The finished product (FP) Zefylti is developed as a biosimilar medicinal product to the reference medicinal product (RMP) Neupogen licensed by Amgen Europe B.V.

The finished product is presented as a solution for injection/infusion use containing 300 µg/0.5 ml (30 million units (MU)/0.5 ml) or 480 µg/0.5 ml (48 MU/0.5 ml) of filgrastim as active substance. It is a clear, colourless or slightly yellowish solution.

Other ingredients are sodium acetate, sorbitol (E420), polysorbate 80 (E433), and water for injections. Nitrogen (Ph. Eur. grade) is used as overlay gas.

The product is available in a Type I glass pre-filled syringe with a permanently attached stainless steel needle in the tip and printed markings for graduations from 0.1 ml to 1 ml (major graduations at 0.1 ml and minor graduations at 0.025 ml up to 1.0 ml) on the barrel.

Each pre-filled syringe contains 0.5 ml solution.

Each pack contains one or five pre-filled syringes, with or without a needle safety guard.

2.3.2. Active Substance

2.3.2.1. General Information

The active substance (AS) filgrastim is a 175 amino acid protein produced by the bacteria *Escherichia coli* (*E. coli*) which harbours the human Granulocyte colony-stimulating factor (GCSF) gene with an N-terminal methionine coding sequence. Filgrastim has a molecular weight of 18,800 Daltons (Da). The r-met-Hu-GCSF contains a N-terminal methionine (different from its native form) that is required for expression in *E. coli*. As filgrastim is produced in *E. coli*, the protein is non-glycosylated and, thus, differs from endogenous GCSF isolated from a human cell. Filgrastim has an α-helical structure with two intra-molecular disulfide bonds formed between cysteine residues at amino acids Cys37 – Cys43 and Cys65 – Cys75, and a single free cysteine at position 18. The disulfide bonds form loop-like structures that maintain the biologically active conformation of the protein. Further structural information is provided in the dossier and is considered acceptable.

2.3.2.2. Manufacture, process controls and characterisation

Manufacturers

Active substance is manufactured at CuraTeQ Biologics Private Ltd, Survey No 77 & 78, Indrakaran, Telengana, India. The GMP certificate for this site was missing and during the assessment a major objection (MO) was raised. Later on, an adequate GMP certificate was provided by the applicant, covering the scope of defined manufacturing and quality control activities. The major objection was considered resolved.

Valid proof of GMP compliance has been provided for the involved sites.

Description of manufacturing process and process controls

The active substance filgrastim (company code BP13) is expressed in genetically modified *E. coli* cells. A detailed description of the manufacturing process is presented in the dossier. AS manufacturing process is divided to upstream and downstream manufacturing steps. During routine manufacturing, critical AS intermediates and AS are not reprocessed or re-worked.

In the upstream manufacturing process, cells are expanded in shake flasks, followed by expansion in fermenter. Cells are separated from the culture medium by centrifugation and are mechanically lysed. The pelleted inclusion bodies containing AS are collected and re-suspended in a buffer and centrifuged.

The downstream manufacturing process starts with thawing, solubilisation and reduction of inclusion bodies. Downstream processing involves filtration and chromatographic purification steps.

The applicant provided a detailed description of the manufacturing process steps that is accompanied by flow charts and tables listing process parameters and IPCs with their classification and acceptable ranges or acceptance criteria. The composition of used buffers, solutions and media is provided. List of reusable chromatographic resins and tangential flow filtration cassettes used during manufacturing are detailed and limits for their maximum permissible reuse cycles have been established based on reusability studies. Sanitisation of chromatographic columns are briefly described for each chromatographic step. Hold times for each process step have been described, and overall time range to complete the upstream and downstream manufacturing process has been defined.

For the manufacture of finished product, the frozen AS is thawed and transferred to the FP facility located in the same building.

The working cell bank (WCB) will be used for commercial manufacturing and only in an unforeseen event, the master cell bank (MCB) would be used to sustain the commercial supply chain.

Overall, an acceptable description of the manufacturing process has been given, and in-process controls are adequately set to control the process.

Control of materials

The majority of the raw materials are of compendial quality. For in-house material, specifications are provided and considered appropriate. No animal-derived materials or materials of human origin are used in the media/buffer preparation or AS manufacturing. All media components are serum free. Certificates of analysis are provided for raw materials and consumables.

Source, history and generation of the plasmid clone has been adequately described.

A two-tiered cell banking system comprised of a master cell bank and a working cell bank is used for filgrastim manufacture. An MCB has been manufactured from a primary cell bank (PCB), and a WCB has been manufactured from a single vial of the MCB. All cell banks were manufactured at CuraTeQ Biologics Private Limited, India. No animal derived materials were used for the manufacture of cell banks. MCB

was used during the manufacture of development, clinical and PPQ (process performance qualification) batches. WCB will be used for the manufacture of commercial filgrastim batches. Manufacture of future WCBs will be initiated when 50 vials of the WCB inventory remains. MCB and WCB have been adequately tested. The testing scheme is considered appropriate. MCB and WCB are stored in cryovials. Specification for filgrastim MCB retesting is provided and is acceptable. Currently proposed testing frequency for MCB is also considered adequate. According to the applicant, all tested MCB batches have met the required release specification.

End of production cell banks (EPCBs) have been formed at the end of the fermentation process and were not extended beyond the normal manufacturing time. This is acceptable as the cells at the end of harvest are expected no longer be dividing and to be loaded with inclusion bodies. EPCBs covered the entire upstream process from seed flask to production fermenter stage. EPCBs have been tested according to ICH Q5D.

Control of critical steps and intermediates

The control strategy has been developed according to relevant ICH guideline Q11. The applicant has discussed in sufficient detail the risk ranking approach to assign critical quality attributes.

Process parameters used during the manufacturing process are categorised into two types: operational parameters and performance parameters. Operational Parameters were defined as input variable or condition of the manufacturing process that can be directly controlled in the process. Risk assessments were performed to identify critical and key process parameters. and a list of identified critical and key parameters is provided. Each parameter has a setpoint and and/or normal operating range (NOR). Performance parameters are divided into in-process controls (IPC) and in-process tests (IPT). In-process tests are used for monitoring and trending of the manufacturing process. Justifications for in-process specification are provided. Overall, the proposed in-process limits are considered appropriate to ensure batch-to-batch consistency. In-process analytical test procedures have been adequately described. Analytical tests used as part of BP13 DS release are adequately validated. Upon request, a validation report for commercial HCP kit was provided during the assessment and was generally considered acceptable. Further information was requested with regards to antibody coverage and on which HCP ELISA kit will be used for commercial manufacture, and based on the provided information the commercial HCP ELISA was considered successfully validated.

Process validation

Results of filgrastim (BP13) validation studies are provided and/or summarised in the dossier.

Commercial scale AS PPQ batches have been manufactured at CuraTeQ Biologics Private Ltd. PPQ data are provided for all the operational parameters (critical process parameters (CPPs), Key process parameters (KPPs), non-CPPs) and performance parameters (IPCs, IPTs) for all AS manufacturing steps. There was no batch failure during validation. No excursion from normal operating ranges (NOR) were reported. In-process tests were within acceptance criteria. All AS results met acceptance criteria. Overall, the process validation data provided indicates that quality of the AS stays consistently at acceptable level when the manufacturing process is operated within specified ranges. A MO has been raised with regards of GMP compliance of the performed AS and FP process validation as it was not considered assured that the PPQ manufacturing process was not affected by the critical deficiencies observed in a previous on-site GMP inspection. This question was clarified during the assessment, and it was considered solved as GMP inspection had zero observations related to the PPQ batch data and the facility related observations were adequately addressed using a comprehensive CAPA approach and verified by the inspectors during the re-inspection in 2024.

Resin reusability studies were performed, and the presented data supports the proposed resin lifetimes. Membrane aging studies were performed to determine the maximum number of cycles. Study reports

were provided and deemed sufficient. Consistent bacterial retention capability of the sterilising-grade filter is demonstrated.

Model virus clearance studies are not required for the AS manufacturing process as it is the product of a bacterial fermentation.

Summaries of hold time studies are provided for process intermediates and buffers used during routine manufacturing. The data support the proposed hold times.

Manufacturing process development

The development of the AS manufacturing process from the process used to produce clinical study material to the proposed commercial process is described. The fermentation process was initially developed at laboratory scales. After completing process development, the process was scaled up to commercial scale. It is declared that no changes were made for the AS manufacturing process throughout the manufacturing process development.

The control strategy was generally developed as according to ICH guidance. Risk assessment was performed for all AS manufacturing process operational parameters to recognise potential CPPs (pCPP) based on their potential impact on one or more critical quality attribute (CQA) and/or performance parameter. Risk assessment reports for upstream and downstream processes including all parameters are provided. pCPPs were further evaluated in a characterisation study. Quality data and statistical analysis of the results for the performed upstream and downstream Process Characterisation (PC) studies are provided.

Based on the PC study results, each pCPP was further classified as a CPP, non-CPP, or key process parameter (KPP). Justification to categorise studied parameters into the CPP, non-CPP and KPP is provided. Ranges (used for commercial manufacture) for the operational parameters are listed in the dossier together with a summary of the control strategy for each parameter. A summary of justifications for acceptance limits of the performance parameters is presented and the proposed limits are considered appropriate.

Overall, the provided information in this section is sufficient.

Characterisation

Extensive product characterisation has been performed using the commercial scale AS batches, in comparison with reference standard. The analytical methods used for AS characterisation are briefly described. According to the applicant, all the methods have been validated/qualified.

Structural attributes included primary structure evaluated by intact mass and peptide map fingerprinting and confirmed by using the LC-ESI-MS/MS technique. Sequence identity, including N-terminal sequence and C-terminal sequence, was confirmed through amino acid sequence analysis by peptide mapping combined with LC-MS and MS/MS methodologies. The physico-chemical characterisation was assessed through size exclusion-high performance liquid chromatography (SE-HPLC) to evaluate size heterogeneity, cation exchange-high performance liquid chromatography (CEX-HPLC) method to determine charge heterogeneity, and reverse-phase high-performance liquid chromatography (RP-HPLC) method to determine structural heterogeneity/ product variants resulting from differences in hydrophobicity. Higher order structure was evaluated by far and near-UV circular dichroism (CD). The functional characterisation of BP13 was evaluated by cell-based MNFS 60 proliferation assay and GCSF-R binding by surface plasmon resonance (SPR).

The results were compared to theoretical values, the primary reference standard (PRS) or the published structure. The intact mass of the AS batches matches the theoretical mass of filgrastim and is identical to the mass observed for the PRS. All the expected peptides for the AS were identified and the molecular

weights of the peptides were matching with their corresponding theoretical molecular weights. The primary sequence and the identity of N-terminal and C-terminal peptides were confirmed. The size variants, product variants and charge variants of BP13 DS batches were comparable to the PRS. AS and PRS exhibited a characteristic alpha-helical secondary and tertiary structure. Relative potency of AS and PRS were comparable.

In principle it is agreed that a panel of state-of-the-art and standard methods have been applied to characterise relevant structural and functional quality attributes. During the assessment, additional characterisation data were generated. The section on characterisation was updated accordingly and is considered appropriate for this type of molecule.

Impurities

The applicant has provided sufficient description of characterisation of process- and product-related impurities in the dossier.

Potential process-related impurities and contaminants were identified. Process clearance data for these impurities and contaminants are provided. It can be concluded that the manufacturing process has a robust capability for impurity removal. Impurity testing has confirmed that these impurities are present at low, consistent levels.

For the downstream process, some components were identified as high risk as per their impact to process and product quality if there is a variation from the recommended quantity and therefore monitored in the process.

Overall, the assessment of process-related impurities through manufacturing process validation and characterisation and AS testing demonstrated that these impurities do not pose a safety risk.

Product-related impurities were controlled through AS specification. The characterisation of product-related impurities is considered sufficient. The characterisation studies performed by the applicant confirmed that the impurity detection methods of RP-HPLC, SE-HPLC, and CEX provided a comprehensive resolution of all expected product variants and serve as essential methods to monitor product quality routinely at AS release.

In-process testing and AS specification ensure control over potential contaminants and adventitious agents.

2.3.2.3. Specification

Specification

Comprehensive panel of specification are set for AS including tests for appearance, visible particles, protein concentration, pH, osmolality, identity via peptide mapping, biological activity by cell-based assay, size heterogeneity by SE-HPLC, structural heterogeneity by RP-HPLC, charge heterogeneity by CEX-HPLC, impurities by SDS PAGE, host cell protein (HCP) by ELISA, host cell DNA (HCD) by qPCR, bioburden, and bacterial endotoxin (BET). Method references to in house-SOPs and Ph. Eur. Monographs/chapters are included, where applicable.

Overall, the proposed test parameters to be included in the AS specification are considered satisfactory and in line with current guidance. All the test parameters have been discussed separately, and justification and batch analysis data have been provided.

Analytical procedures and validation of analytical procedure

Release and stability testing of AS are performed at CuraTeQ (India). Summary of analytical methods is provided in the dossier. Description of the in-house methods is considered satisfactory. Reference to compendial methods is made and considered acceptable.

Physical appearance, clarity and colour of the solution are tested according to Ph. Eur. 2.2.1 and Ph. Eur. 2.2.2. Visible particles are analysed according to Ph. Eur. 2.9.20. The analytical procedure for measuring pH is performed in accordance with Ph. Eur. 2.2.3 and USP <791> and the method for osmolality measurement is determined according to Ph. Eur. 2.2.35 and USP <785>. According to the applicant, these compendial analytical procedures have been verified to establish AS suitability during routine analysis.

For endotoxin testing, the Ph. Eur. 2.6.14 and USP <85> limulus amoebocyte lysate (LAL) test using the gel-clot method was implemented. Validation report for BET by Gel Clot method was provided and is considered appropriate. The results demonstrate that the method is suitable for AS sample matrix. However, the applicant was encouraged to consider the feasibility of transitioning to Ph. Eur. 2.6.32 Test for bacterial endotoxins using recombinant factor C, thus eliminating the need for horseshoe crab derived material and to follow ICH Q10 (4.2(b)). Detailed plans on the effort to develop and implement an endotoxin assay based on recombinant Factor C has been provided during the assessment (REC).

Bioburden was established according to Ph. Eur. 2.6.12 using the membrane filtration method. Overall, the recovery of challenge organisms was appropriately performed, and the results demonstrated that the method is suitable for its intended use.

The non-compendial methods Protein concentration by A280, peptide mapping by RP-HPLC, SE-HPLC, CEX-HPLC, host cell DNA qPCR, host cell protein ELISA and potency assay used for DS testing are sufficiently described. The system suitability, assay and sample acceptance criteria are found suitable to confirm that the methods are performing as expected during release testing. The non-compendial in-house analytical procedures were validated as per ICH Q2 (R1) guidelines. Approved protocols and validation reports for each analytical method were provided. In general, relevant parameters have been assessed and the presented verifications and validation reports indicate suitability of the analytical procedures for their intended use. Upon request, validation report for commercial Cygnus HCP ELISA kit has been provided and was considered generally acceptable. Additional data and clarification were provided during assessment and based on that the commercial HCP ELISA is considered successfully validated.

Batch analysis

Batch analysis data are provided for AS (BP13) batches, all of which were manufactured at CuraTeQ, India facility. Batch information include batch scale, manufacturing date and batch usage. All batches were tested as per the specifications in place at the time of release. All results met the pre-determined specifications that were in place at the time of release.

Reference standard

Internal reference standard (IRS) was established and characterised.

Primary reference standard (PRS) was produced, and adequately characterised results are provided and are adequately discussed by the applicant. Re-qualification of the PRS is performed annually, and the stability of the reference standard is monitored during annual re-qualification. Real time stability data are provided, and all acceptance criteria were met. The stability testing plan is acceptable.

Secondary reference standard (SRS) will be prepared from commercial batches and used as reference standard for future commercial batch release and stability testing. A plan to establish an SRS was

submitted. The newly prepared SRS will be qualified against the primary reference standard and against WHO NIBSC. The applicant's proposal for preparation and qualification of the SRS are acceptable

Certificates of analysis are provided for the designated certified reference materials.

Container closure

AS is filled and stored in PETG (polyethylene terephthalate co-polyester – glycol modified) bottles with HDPE (high-density polyethylene) closures. Appropriate in-house specifications are provided. Bottles are released based on CoA that is issued by the container closure supplier.

To demonstrated suitability of the containers, a simulated leachable study has been performed by the applicant. Leachable samples were evaluated via HS-GC-MS, GC-MS and LC-UV-MS, and no compounds were detected above the chosen analytical evaluation thresholds in the leachables solutions. Extractable/leachable studies have been performed for final AS filter and storage container with respective reports provided. Suitability of container closure system and AS has been confirmed by stability studies.

2.3.2.4. Stability

A shelf-life of 24 months at the recommended -20 ± 5°C real-time storage condition is claimed for the AS.

To support this claim, the applicant has provided stability data at -20 ±5°C (long-term), +5 ± 3°C (accelerated), and +25 ± 2°C, 60 ± 5% RH (stress) storage conditions. Currently, 24 months of real-time stability data at long-term storage condition, six months of accelerated stability and 30 days of stress stability data are available. Stability study samples are stored in PETG bottles representative of the AS container closure system with same materials of construction.

The stability protocol is in line with the relevant ICH guidelines and relevant quality attributes for AS are considered. The methods that were chosen for the stability study were considered as stability indicating.

In general, the presented data showed good stability in long-term, accelerated and stress stability conditions. Stability testing under accelerated and stress conditions indicate that AS material is stable under higher temperatures. No out-of-specification values or significant trends over time have been observed. Based on this data, the proposed shelf-life of 24 months is considered acceptable to support the overall stability conclusion.

Freeze-thaw studies have been performed allowing up to three freeze-thaw cycles for AS. No photostability studies have been performed for AS.

Post-approval stability commitment has been provided. Furthermore, a commitment is provided to inform the competent authorities immediately in case of out-of-specification results.

Overall, the stability results indicate that the active substance is sufficiently stable and justify the proposed shelf life of 24 months at the recommended -20 ± 5°C in the proposed container.

2.3.3. Finished Medicinal Product

2 . 3 . 3 . 1 . Description of the product and pharmaceutical development

Description of the product

The finished product is a clear, colourless or slightly yellowish solution, presented as a solution for injection/infusion use, containing 300 µg/0.5 ml or 480 µg/0.5 ml of filgrastim as active substance. The product is available in a Type I glass pre-filled syringe with a permanently attached stainless steel needle in the tip and printed markings for graduations from 0.1 ml to 1 ml (major graduations at 0.1 ml and minor graduations at 0.025 ml up to 1.0 ml) on the barrel.

Each pre-filled syringe contains 0.5 ml solution.

Other ingredients are sodium acetate, sorbitol (E420), polysorbate 80 (E433), and water for injections. Nitrogen (Ph. Eur. grade) is used as overlay gas.

No overage is applied. Sufficient information on the FP components, their function and the references are presented.

Formulation development

The FP formulation has been developed in a similar way to the reference medicinal product Neupogen. Comparable degradation pathway, degradation products, and degradation rates of Zefylti and EU-Neupogen have been demonstrated in a comparative side-by-side forced degradation study. Further, excipient range establishment study employing varying excipient concentrations under thermal stress, accelerated and real-time conditions has been performed with data demonstrating FP stability with the studied excipient concentrations. The establishment of the specification range for pH was not directly addressed during formulation development. However, the (design of experiments (DOE) study so far shows robust quality attributes when varying excipients and keeping the pH stable. This indicates that the chosen pH range could be regarded suitable for stability of the FP. However, the excipient range establishment study is still ongoing and, an update of results should be provided when available (REC). Albeit limited, the overall approach to formulation development can be accepted.

Target product profile of the product is presented in the dossier. There are no differences in formulation or manufacturing process between clinical and commercial batches. No excipients of human or animal origin are used for manufacture of FP. No novel excipients are used in the formulation of FP. Examples of certificates of analysis and TSE/BSE certificates are provided for the excipients. No overage is applied in the formulation.

Manufacture process development

The manufacturing process entails thawing of AS, preparation and filtration of formulation buffer, preparation of formulated bulk solution, bioburden reduction filtration, sterile filtration, PFS filling, stoppering, visual inspection and storage of naked PFS, labelling, plunger rod fixation, needle safety guard assembly, secondary packaging and storage. Sterility of the FP is achieved through aseptic filtration.

The same manufacturing process was used from the development to commercial manufacturing. No substantial changes were made to the manufacturing process parameters, process controls, sterilisation procedure or equipment from the development and clinical batches to the PPQ and commercial batches. The results for product quality attributes for the clinical and PPQ batches are found comparable and meet the acceptance criteria.

To evaluate the impact of FP manufacturing process operating parameters on the product CQAs, the applicant has performed a risk management evaluation. Justification for the classification of the operating parameters as CPP or KPP has been provided upon request. In addition, upon request, additional characterisation data has been provided. Set points and normal operating ranges (NOR) for the process parameters are defined. Filter validation studies were performed, and results indicate that there is no impact on quality attributes of the FP. Extractable safety risk assessment for the filters was appropriately performed. Maximum daily exposure of extractables above the reporting threshold are below the substance specified permitted daily exposure. Also, all extractables below the reporting threshold do not exceed the threshold of toxicological concern.

Container closure system

The proposed primary packaging materials consists of a 1.0 ml Type I borosilicate graduated glass pre-fillable syringe (PFS) affixed with a staked stainless-steel needle capped with an elastomeric needle shield and a polypropylene rigid needle shield and sealed with a rubber plunger stopper with fluoropolymer barrier film coating. After filling, the PFS are assembled with a plunger rod and, in case of the PFS with needle safety guard (NSG), a passive ready-to-use needle guard is affixed. Drawings and representative vendor conformity certificates have been provided. The product contact components (i.e., pre-fillable syringe and plunger stopper) are pre-sterilised (ethylene oxide / ionisation radiation) and are supplied as ready-to-use. The product contact components are accepted for FP manufacturing based on an in-house specification and a valid certificate of conformance from the supplier. The materials are compliant with Ph. Eur. The choice of materials for primary packaging is justified and the compatibility and safety are discussed. Particulate formation in FP due to release of silicone oil from the siliconised inside of the PFS glass barrel has been demonstrated to be within acceptance limits at long term storage conditions and have no impact on FP quality and potency. The components of the primary packaging material have been properly described, and the materials of the containers and closures appear to comply with the applicable quality requirements.

The same container closure system is used for both FP strengths. No changes are made to the container closure system during the course of process development. The target fill is considered adequate for the deliverable single dose of 0.5 ml.

Extractables and leachables studies were performed using appropriate analytical methods. The choice of the solvents is justified. No compounds were detected in the extractable study or the leachable study that are of potential safety concern for patients or that were above the analytical evaluation thresholds and reporting limits for up to 36 months.

Device functionality has been demonstrated throughout the entire shelf-life. The functionality of the PFS with NSG has also been demonstrated using several FP batches exposed to vibration, temperature excursion, and agitation during air shipment and package drop test. Functionality testing is part of the release and stability specification.

Microbiological attributes

No preservatives are used in the manufacture since PFS is intended for single use. The product contact container closure components are pre-sterilised and supplied as ready-to-use. Sterility of the FP is ensured by sterile filtration, aseptic processing and by the integrity of the container closure system. The aseptic manufacturing process (filter sterilisation) has been validated. The integrity of the container closure system was evaluated using both dye ingress and microbial ingress tests; respective results confirm the suitability of the primary container closure system. Appropriate controls are in place at FP manufacturing. Taken together, the measures set are considered sufficient to ensure microbiological integrity of the FP.

Compatibility

The FP is stored in a single-use pre-filled syringe administered as a single dose either via subcutaneous injection or as intravenous infusion diluted in 5% glucose solution with optional addition of human serum albumin (HSA) depending on the required concentration. To demonstrate the compatibility and physicochemical stability of FP with the materials used for administration to patients, and that the infusion solution containing the FP does not support the growth of micro-organisms up until 36 hours, the applicant has conducted in-use stability studies. Based on the results, 5% glucose and 20% HSA do not support microorganism growth. No incompatibilities between FP and glass bottle or polypropylene container were observed for the tested parameters.

2.3.3.2. Manufacture of the product and process controls

Manufacturers, batch formula, manufacturing process and critical steps

Name, address, and responsibilities of manufacturers involved in the manufacture, in-process and quality control and stability testing of FP are listed.

Valid GMP certificates covering the indicated responsibilities for the sites involved are provided and their GMP compliance is thus confirmed. An EU release test site(s) for biological, chemical and physical tests was not registered in the dossier and this issue was raised as MO. During the assessment, the applicant adequately addressed this question by registering a site for these activities and providing valid GMP certificates and method transfer validation reports. An appropriate qualified person declaration was provided.

Batch formula is provided, including a list of excipients. No AS pooling is performed to manufacture the FP. Batch numbering system of commercial FP is clearly described.

The FP manufacturing process consists of thawing of AS, preparation of formulation buffer, formulation of bulk FP, bioburden reduction filtration of formulated bulk, aseptic filtration, filling, stoppering, visual inspection, naked PFS storage, labelling, plunger rod insertion, needle safety guard/safety device assembly (only for PFS with NSG), secondary packaging, and storage. Manufacturing process flow chart including process parameters and in-process tests, as well as narrative description for each step of the FP manufacturing process are presented. All filled PFS are 100% visually inspected and defect PFS are rejected. Critical- and key process parameters were defined and normal operation ranges indicated. For the control of critical steps, suitable in-process controls (IPCs) and in-process tests including acceptance criteria were established.

Analytical methods and validation of analytical methods used in FP in-process testing are briefly mentioned.

There are no intermediates in the FP manufacturing process. The hold times were validated. Reprocessing is not foreseen for the FP manufacturing process.

Process validation

Media fill is demonstrated.

Data on sterile filter validation are provided and considered acceptable.

PPQ was performed for FP batches per strength (300 µg/0.5 ml and 480 µg/ 0.5 ml). FP release specifications, process parameters (CPP, KPP) and performance parameters (IPC, IPT) were monitored. The provided data demonstrate that when operating within the proposed normal operating ranges, the performance controls meet relevant quality criteria. Furthermore, data from post-PPQ batches confirm consistency of the manufacturing process. An MO was raised in regards of GMP compliance of the

performed AS and FP process validation, which was adequately addressed during the assessment, as stated in the AS section above.

Cumulative processing time for PPQ FP batches are provided.

Hold time validation has been performed to establish maximum acceptable holding times for FP manufacturing process steps. The provided data for FP batches support the proposed hold times.

Shipping validation has been conducted. Shipping validation showed comparable release test results before and after shipping. All results conformed to their specification. Thermal cycling as well as mechanical stress (agitation) studies were performed to support eventual issues during transportation. Based on the provided data, the shipping conditions do not adversely impact product quality, device functionality or integrity of packaging components. Information on shipping validation is sufficient.

2.3.3.3. Product specification

Specification

Comprehensive panel of release and stability specification are set for FP including tests for appearance, visible and sub-visible particles, protein concentration, pH, osmolality, extractable volume, identity via peptide mapping, biological activity by cell-based assay, purity by size (SEC, RP-HPLC), charge heterogeneity by CEX, sterility, bacterial endotoxin, container closure integrity, PS80, and PFS functionality tests. A reference to in house-methods or Ph. Eur. are included. It is noted that data for Identity by peptide mapping are not provided for stability studies. This is acceptable.

Justification of specification are provided. In general, the acceptance criteria set for each QA are considered acceptable and appropriately justified by clinical batch data and supported by biosimilarity characterisation data or compendial requirements. In addition, the impurity release acceptance criteria for structural heterogeneity by RP-HPLC are set according to the minimum quality requirements for impurities based on the monograph for filgrastim for injection (*07/2019:2848 corrected 11.0*).

In-house analytical procedures used for both FP and AS release and stability testing are described and their validation included in the dossier. Compendial method Ph. Eur. references are provided. Method verification and validation and method transfer validation reports have been provided.

Batch analysis data were provided. All acceptance criteria were met and no significant changes between batches were observed in any of the quality attributes.

The potential presence of elemental impurities in the finished product has been assessed on a risk-based approach in line with the ICH Q3D Guideline for Elemental Impurities. Batch analysis data using a validated ICP-MS method was provided, demonstrating that each relevant elemental impurity was not detected above 30% of the respective PDE. Based on the risk assessment and the presented batch data it can be concluded that it is not necessary to include any elemental impurity controls. The information on the control of elemental impurities is satisfactory.

A risk evaluation concerning the presence of nitrosamine impurities in the finished product has been performed (as requested) considering all suspected and actual root causes in line with the "Questions and answers for marketing authorisation holders/applicants on the CHMP Opinion for the Article 5(3) of Regulation (EC) No 726/2004 referral on nitrosamine impurities in human medicinal products" (EMA/409815/2020) and the "Assessment report- Procedure under Article 5(3) of Regulation EC (No) 726/2004- Nitrosamine impurities in human medicinal products" (EMA/369136/2020). Based on the information provided it is accepted that no risk was identified on the possible presence of nitrosamine

impurities in the active substance or the related finished product. Therefore, no additional control measures are deemed necessary.

Reference materials

Reference standard used for finished product testing is the same as for the active substance.

2.3.3.4. Stability of the product

The applicant claims a shelf-life of 36 months at 5 ± 3°C. Stability data for the long-term real-time conditions are available. In addition, six-month stability data for accelerated conditions and one month data for stress conditions are available.

The stability studies are carried out under the conditions described in the ICH guideline. The results on clinical batches and PPQ batches meet all the acceptance criteria under long-term stability condition. Overall, the provided data is supportive of the 36-month shelf-life claim.

Real-time stability data at 24-month timepoint has been provided for FP PPQ batches. All the results met the acceptance criteria. However, as 36-month shelf life is proposed and as charge variants, Polysorbate 80 and device functionality testing have not been studied for the primary stability studies but are included in the PPQ stability studies with up to 24-month data provided, the applicant is recommended to provide the 36-month stability data for the FP PPQ batches (REC).

Device functionality test results at real time conditions met specifications up to 36 months. Functionality testing on surrogate solution batches is still ongoing (12-month time point). It is agreed that the dataset supports functional stability of the device over 36 months at real time conditions.

Physiochemical in-use stability for infusion has been demonstrated in the thermal cycling study for 24 hours at 25 ± 2°C and 5 ± 3°C. Based on the provided photostability data, FP should be stored protected from light, which is appropriately reflected in the SmPC.

Appropriate post-approval stability protocol and stability commitment are provided.

Based on available stability data, the shelf-life of 3 years at 5 ± 3°C and protected from light, as stated in the SmPC, are acceptable.

2.3.3.5. Biosimilarity

Similarity assessment

A stepwise approach to demonstrate the similarity between FP and EU-Neupogen has been presented. First, a target product profile was assessed, next the quality attributes of FP were classified based on risk ranking to recognise the CQAs. Then, based on the criticality assignments, a statistical approach for biosimilarity data analysis was selected. The description of the applied risk assessment tools is considered adequate and in line with regulatory expectations. The tier ranking of quality attributes based on assessed criticality score is considered appropriate.

Justification for statistical approaches were provided, and these are considered acceptable as supportive evidence for biosimilarity.

The analytical similarity study includes data from Zefylti FP batches (manufactured at-scale) and EU-Neupogen batches. Zefylti FP batches originated from different AS batches. Ages of the batches at the time of analytical testing vary between 2 to 19 months whereas for EU-approved Neupogen ages are 10

to 24 months. Considering the highly stabile nature of the RMP and Zefylti at the recommended storage condition, the slight age difference is not considered significant. The clinical Neupogen batch is confirmed to be sourced from Germany. Six out of nine of the Neupogen batches are sourced from UK. As the development of Zefylti as biosimilar has started before the Brexit, the use of the UK-sourced Neupogen batches as RMP can be principally accepted. Two of these UK sourced batched are released after or at the time of the Brexit and will not be considered as part of the analytical similarity assessment. Based on the analytical similarity data excluding these two batches, the conclusion on similarity will not change. It should be noted that the number of EU sourced RMP batches is rather limited; however, taking into account that filgrastim is a rather simple protein molecule with no extensive post-translational modifications the somewhat reduced number of RMP batches is acceptable.

Overall, the proposed biosimilarity approach follows the general principles outlined in the guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance.

Method qualification

To demonstrate biosimilarity between Zefylti and the EU-sourced Neupogen, the applicant has presented an extensive similarity exercise using sensitive and orthogonal methods. According to the applicant, all the assays used in the biosimilarity are demonstrated to be suitable for their intended purpose. Batch release analytical methods are validated, and other methods have been qualified. Assay qualification summaries are provided.

Summary of results

Summary of the results are provided.

Table 1 - Analytical Similarity Assessment between Zefylti and EU-Neupogen

Product Characteristic	**Product Quality Attribute**	**Conclusion and Key Findings**
Protein Content/Dose	Protein Concentration	Protein concentration for all the BP13 batches is similar to the EU-approved Neupogen batches and within the release acceptance limits for both presentations
Primary Structure	Amino acid composition	The extinction coefficient results for BP13 batches and EU-approved Neupogen batches are similar, confirming that the amino acid composition of both products is identical.
	Molecular Mass (Intact)	The primary structure, in terms of intact mass and amino acid sequence, including the N- and C-terminal sequence, is identical between BP13 and EU-approved Neupogen batches.
	Amino acid sequence	
	N- and C-Terminal Sequence	
	pI	A similar pI of the main peak is observed in BP13 and EU-approved Neupogen.
	Non-canonical amino acids	The non-canonical relative percentages are similar between BP13 and EU-approved Neupogen.
	Identity	The visual profile assessment (band profiles) and molecular

Product Characteristic	Product Quality Attribute	Conclusion and Key Findings
		weight between the BP13, EU approved Neupogen is identical.
Size Heterogeneity	Monomer, Aggregates, High Molecular Weight species	The percentage of HMWs and the main peak of BP13 batches were within the quality range of the EU approved Neupogen. Qualitative profiles are highly similar, and molecular weight band distribution is similar among BP13 and EU-approved Neupogen batches.
	Sub-visible Particles	Measurements demonstrated a low number of sub-visible particles in BP13 compared to the EU-approved Neupogen.
Structural Heterogeneity	Purity	The hydrophobic variants (pre- and post-peak) and the Percent main peak of BP13 batches are similar and within the range of EU-approved Neupogen batches.
Charge Heterogeneity	Acidic isoforms, Basic isoforms, Neutral isoform (Main Peak)	Overall, the percentages of the main peak and acidic variants of BP13 batches fall within the quality ranges (established) using data from EU-approved Neupogen batches.
Post Translational Modification (PTM)	Oxidation	The oxidation and deamidation levels of both products are similar.
	Deamidation	
Higher Order Structure	Secondary and Tertiary Structure	The higher order structure of BP13 and the EU-approved Neupogen was evaluated by an array of orthogonal methods and are indistinguishable from the EU-approved Neupogen batches.
	Molar Mass and Hydrodynamic Radii	The size variant profiles are similar in both products.
	Free Cysteine	The free cysteine levels are found to be similar in both products.
	Di-Sulphide Bond Assignment	The data observed indicates the same disulphide bond presence in BP13 and EU-approved Neupogen batches.
Functional Characterisation	Potency	The relative potency values of BP13 and EU-approved Neupogen batches are similar, and the Equivalence testing criteria are met.
	Binding Activity	Relative binding affinities of all the BP13 batches fall within the quality ranges, and thus, both products are considered similar in terms of their binding affinity
	Signalling	Data is comparable between BP13 and EU-approved Neupogen batches.
Immunogenicity	Innate Immune Response	Components were absent in the BP13 drug product and EU-approved Neupogen batches,

Product Characteristic	Product Quality Attribute	Conclusion and Key Findings
		indicating no potential to induce innate immunity
		BP13 and EU-approved Neupogen batches induced similar responses in donors
	Adaptive immune response	There is no evidence of increased immunogenicity risk in the BP13 batches compared to the EU-approved Neupogen batches

Physicochemical properties, FP attributes

Biosimilarity in terms of protein content per dose is agreed. In addition, head-to-head assessment of deliverable/extractable volume, osmolality, pH and excipients content have been performed. Slight differences were observed most probably due to differences in excipient content.

Primary structure

Primary structure was characterised for molecular mass, amino acid composition, sequence coverage/peptide mapping, amino acid sequence and non-canonical amino acids, pI, and identity. The results demonstrate Zefylti to be identical to EU-approved Neupogen in terms of primary amino acid sequence.

Complete sequence of N-Terminal and C-terminal peptides were confirmed to be identical to that of EU-Neupogen.

Isoelectric Point (pI) were found to be similar in both products. Extinction coefficient has been determined. Overall, Zefylti is found to be identical to EU-Neupogen in terms of primary structure.

Molecular heterogeneity

Molecular heterogeneity was characterised for size and charge heterogeneity, structural heterogeneity and post-translational modifications. Results indicated similar structural, size, and charge heterogeneity between the products.

Higher order structure

Higher order structure was characterised for secondary and tertiary structure, molecular mass and hydrodynamic radii, free cysteines and disulphide bonds. Comparable profiles were observed indicating similar secondary structures. The assessment of the profiles for tertiary structure of Zefylti is similar to that of EU-Neupogen.

Functional characterisation

Zefylti functional properties were characterised and found comparable.

Comparative stability

To understand the effect of product degradation profiles, the applicant has performed comparative thermal stability and forced degradation studies with as closely as possible age-matched Zefylti and EU-Neupogen batches. Overall, the degradation pathway, degradation products, and degradation rates were comparable.

Finished product attributes

In addition to the extensive characterisation of physicochemical and biological properties, FP attributes such as deliverable/extractable volume, osmolality, pH and excipients content were tested in a head-on study to demonstrate similarity between the products.

Conclusion

Based on the provided data, Zefylti is considered as a biosimilar to EU-Neupogen with some post authorisation measures (REC) agreed (as below).

2.3.3.6. Post approval change management protocol(s)

Not applicable.

2.3.3.7. Adventitious agents

Filgrastim is expressed in *E. coli*. No raw materials of biological or animal origin are used. Defined medium components are used. It is therefore agreed that viral risk and TSE risk are negligible. All raw materials are verified to meet the vendor specification for microbial safety parameters before use. Considering the nature of the product, adventitious agents safety evaluation has been satisfactorily performed.

2.3.3.8. GMO

Not applicable.

2.3.4. Discussion on chemical, pharmaceutical and biological aspects

Zefylti (BP13) is developed as a filgrastim biosimilar to the reference medicinal product EU-Neupogen.

The manufacturing processes for the active substance and finished product reflects a standard manufacture of filgrastim products. During the assessment one MO was raised and subsequently adequately addressed, as a valid GMP certificate has been provided. Two more major objections were raised, one on the EU release test sit(s)e for biological, chemical and physical tests that had not been registered in the dossier and another one regarding the GMP compliance of the performed AS and FP process validation. These questions were all adequately addressed during the assessment.

The AS and FP manufacturing processes, process controls, process development and process validations as well as raw and starting materials used for the manufacture have, overall, been appropriately described. The results of tests carried out indicate consistency and uniformity of important product quality characteristics, and these in turn lead to the conclusion that the product should have a satisfactory and uniform performance in clinical use.

Comprehensive panels of release specifications are set for AS and FP. Analytical methods have been appropriately validated.

Reference standards are sufficiently described and characterised. The proposed procedures for re-qualification and qualification of new reference standards are acceptable. A comprehensive risk assessment on impurities has been provided and found acceptable.

The FP is a sterile solution for injection/infusion in two strengths - 300 µg/0.5 ml and 480 µg/0.5 ml. The excipients are of Ph. Eur. quality. The FP is packed in pre-filled syringe with hypodermic needle with or without needle guard for safety. The single-use device components and medicinal product form a

single integral product. A notified body opinion on the conformity of the integral device part is provided and considered acceptable.

The similarity between Zefylti and the reference product, EU-Neupogen has been addressed in a comprehensive comparability exercise. In general, from a quality perspective, the results derived from the biosimilarity exercise support the biosimilarity claim for physicochemical, structural and functional attributes.

The applicant has agreed to three post authorisation recommendations, which are related to excipient range establishment study, endotoxin assay and 36-month stability data for the FP PPQ batches.

To conclude, the quality part of the dossier is sufficient and adequate, with three post authorisation measures (REC) agreed (as below).

2.3.5. Conclusions on the chemical, pharmaceutical and biological aspects

The quality of this product is considered to be acceptable when used in accordance with the conditions defined in the SmPC. Physicochemical and biological aspects relevant to the uniform clinical performance of the product have been investigated and are controlled in a satisfactory way. Data has been presented to give reassurance on viral/TSE safety.

2.3.6. Recommendation(s) for future quality development

In the context of the obligation of the MAHs to take due account of technical and scientific progress, the CHMP recommends the following points for investigation:

Proposed list of recommendations:

Description of post-authorisation measure(s)
• *For the excipient range establishment study, the applicant is recommended to provide an update of results (36 months data) once available (by September 2025).*
• *The applicant is recommended to proceed with the assay development and implement an endotoxin assay based on recombinant Factor C.*
• *The applicant is recommended to provide the 36-month stability data for the FP PPQ batches once available (by March 2025).*

2.4. Non-clinical aspects

2.4.1. Pharmacology

2.4.1.1. Primary pharmacodynamic studies

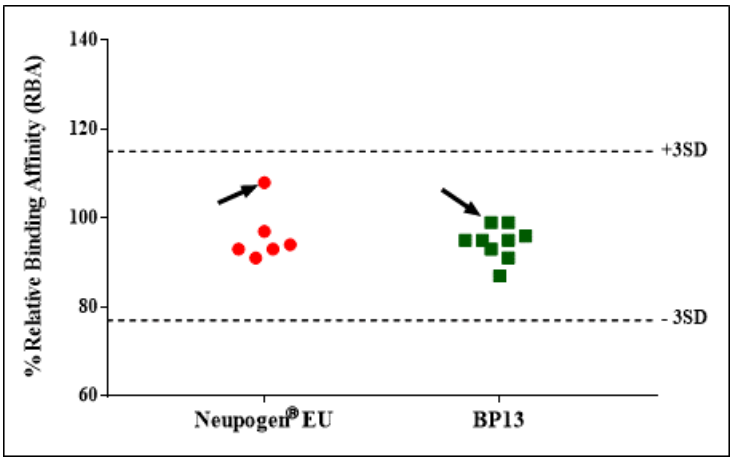
Comparative *in vitro* assays included binding to G-CSF receptor on a chip through SPR, cell proliferation, and phospho-STAT3 signalling activity as a downstream pathway following filgrastim and G-CSFR binding to demonstrate similarity of BP13 and Neupogen pharmacodynamic profile.

Additional *in vitro* studies included assessment of immunogenicity presented under Toxicology/Immunotoxicity.

G-CSFR binding

BP13 and Neupogen were similar in their binding affinity to G-CSF receptor. The relative binding affinities for BP13 lots were from 87% to 99%, falling within the calculated quality range (77 – 115%).

Figure 1. G-CSFR binding



Proliferation

BP13 and Neupogen were considered similar according to a rhG-CSF adapted mNFS-60 cell-based proliferation assay. BP13 and Neupogen lots had relative potency of 91 ± 4 % and 89 ± 6 % (average ± SD), respectively. The observed difference of means (-2.1) and upper and lower 95% confidence intervals were within the defined equivalence acceptance criteria (± 8.9).

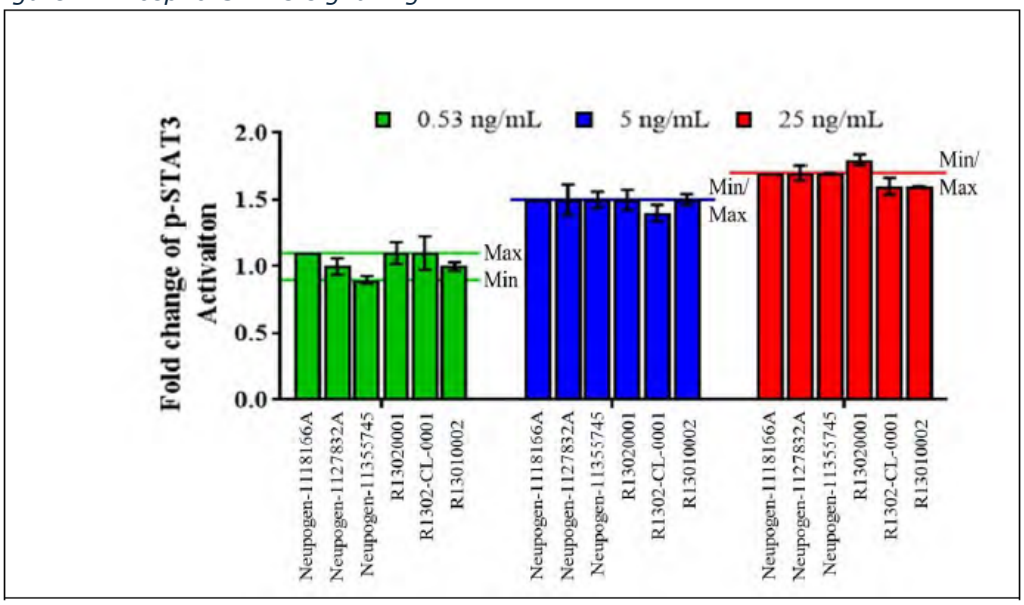
Table 2. Proliferation of mNFS-60 cells

Products	# of Lots	Relative potency (%)		Equivalence acceptance criteria (EAC)	Comparison of difference of means between relative potency values from Neupogen and BP13 using TOST analysis		
		Average	SD		Difference of means	Upper 95% CI	Lower 95% CI
BP13	9	91	3.8	8.9	-2.1	3.0	-7.2
Neupogen	8	89	5.9				

Phospho-STAT3 signalling

Phospho-STAT3 signalling profiles (based on the visual comparison) of BP13 and Neupogen were similar in mNSF-60 cells.

Figure 2. Phospho-STAT3 signalling



2.4.1.2. Secondary pharmacodynamic studies

No secondary pharmacodynamic studies were conducted, in accordance with the relevant EMA Guidelines for similar biological medicinal products.

2.4.1.3. Safety pharmacology programme

No safety pharmacodynamic studies were conducted, in accordance with the relevant EMA Guidelines for similar biological medicinal products.

2.4.1.4. Pharmacodynamic drug interactions

Not applicable.

2.4.2. Pharmacokinetics

No pharmacokinetic studies were performed, in accordance with the EMA Guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: non-clinical and clinical issues (EMEA/CHMP/BMWP/42832/2005 Rev1) and the Guideline on similar biological medicinal products containing recombinant granulocyte-colony stimulating factor (EMEA/CHMP/BMWP/31329/2005 Rev1).

2.4.3. Toxicology

No *in vivo* toxicology studies were performed, in accordance with the relevant EMA Guidelines for similar biological medicinal products. The applicant conducted three *in vitro* immunotoxicity comparative studies of BP13 and the reference medicinal product (RMP) Neupogen.

Immunotoxicity

Three *in vitro* immunogenicity studies were conducted which evaluated the potential agonistic effect on various receptors known to recognise pathogen associated molecular patterns (Toll-like receptors, TLR), Peripheral blood mononuclear cells (PBMC) activation by the release of cytokine/chemokines (GM-CSF, IFN-γ, IL-1β, IL-2, IL-6, IL-10, IP-10, MCP-1, MIP-1α, MIP-1β, RANTES, TNFα) and effects on T-cell proliferation (DC:CD4 proliferation assay). In these assays, three BP13 batches (R1302-CL-0001, R1302-CL-0003 and R13020001) were compared against three baches of EU-Neupogen (118166A, 1119201 and 1127832A).

- **Effects on Toll-like receptors**

The three lots of BP13 and Neupogen did not activate the hTLR2, hTLR3, hTLR4, hTLR5, hTLR7, hTLR8, or hTLR9 reporter cell lines indicating similar behaviour in their potential agonistic effect on the Toll-like receptors known to provoke innate immune response.

- **Effects on cytokine/chemokine release in human PBMC**

The effects on cytokine/chemokine (GM-CSF, IFN-γ, IL-1β, IL-2, IL-6, IL-10, IP-10, MCP-1, MIP-1α, MIP-1β, RANTES, TNFα) release induction was analysed in PBMC from healthy human donors. Any batch to be determined as significantly immunogenic, should had at least 2-fold increase in response along with a p-value of < 0.05 compared to Blank sample. Based on the results (Table 3, Table 4 and Figure 3) it was concluded that BP13 was similar to EU-Neupogen in the risk of activating an immune response.

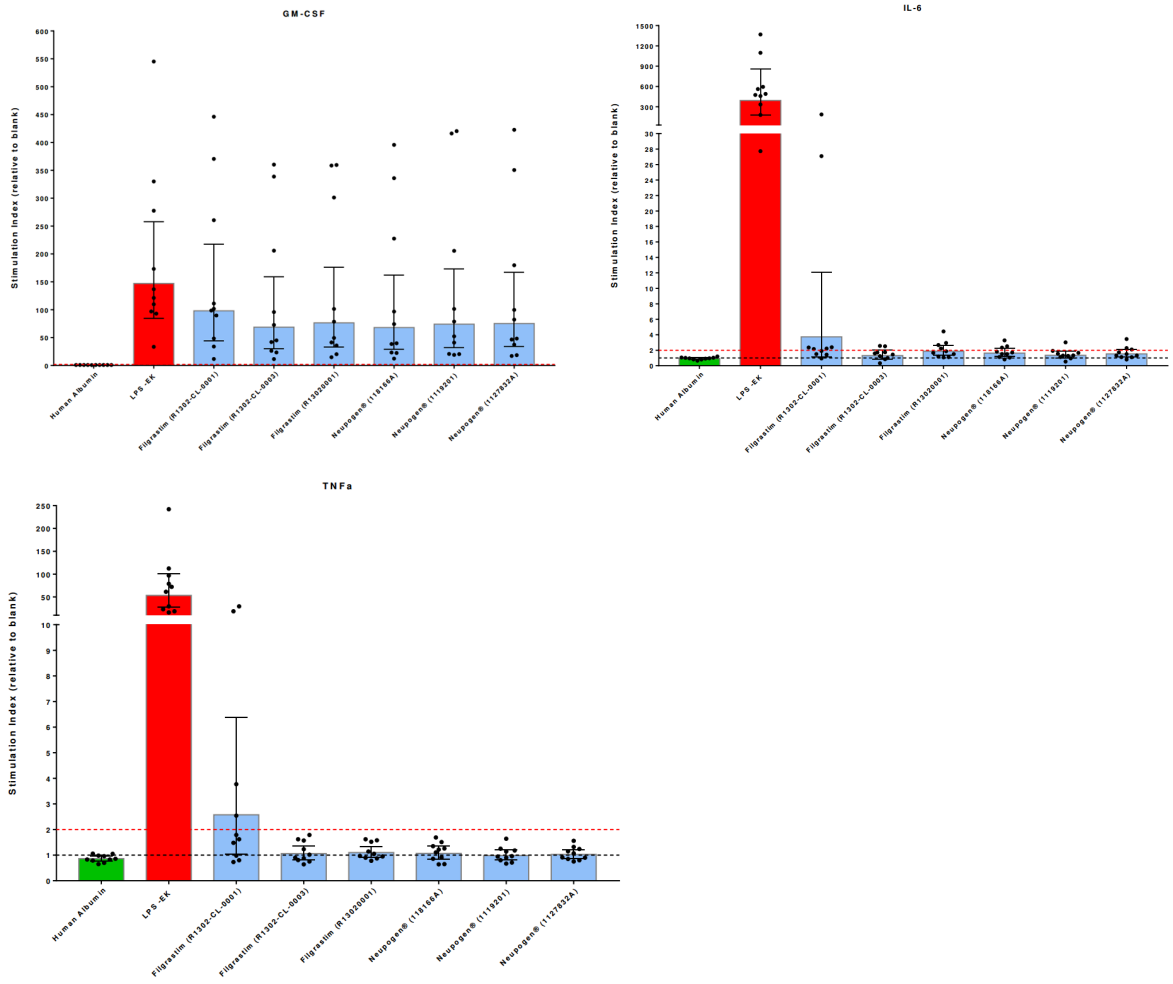
Table 3. Cytokine/chemokine responses

Sample	Number of donors with significant increase in cytokine/chemokine in response to stimulus with a given batch											
	GM-CSF	IFN-γ	IL-10	IL-1β	IL-2	IL-6	IP-10	MCP-1	MIP-1α	MIP-1β	RANTES	TNFα
Blank	1.18	20.02	2.20	3.61	5.62	13.87	831.98	212.98	46.59	287.58	296.05	1425.96
Human Albumin	1.09	19.06	2.01	3.31	4.69	12.95	782.48	220.58	39.33	263.95	284.45	1227.26
LPS -EK	173.18	471.16	102.48	2056.34	78.39	5461.71	3118.23	263.50	232.65	2170.68	1530.04	76123.39
Filgrastim (R1302-CL-0001)	115.43	70.51	40.59	9.91	12.57	51.83	935.87	296.35	56.65	378.87	372.37	3677.60
Filgrastim (R1302-CL-0003)	80.87	32.86	28.29	3.92	7.50	18.20	755.06	269.56	30.95	220.70	231.29	1508.78
Filgrastim (R13020001)	90.03	34.15	28.42	4.98	7.46	25.98	784.76	275.98	34.63	224.69	241.66	1570.14
Neupogen® (118166A)	80.10	34.25	27.98	4.47	8.63	22.66	799.59	264.61	33.57	229.73	240.19	1521.09
Neupogen® (1119201)	87.18	33.22	27.86	3.81	6.81	18.63	707.58	256.39	27.31	223.68	234.93	1404.38
Neupogen® (1127832A)	88.54	34.28	27.03	4.31	7.34	21.32	786.97	281.37	29.40	240.59	246.68	1464.53

Table 4. Mean cytokine response with exclusion of two high responding donors for R1302-CL-0001 batch

Cytokine	Cytokine response for R1302-CL-0001 (pg/mL) n=10	Cytokine response forR1302-CL-0001 (pg/mL)/ Excluding donors
GM-CSF	115.53	94.09
IL-10	40.59	29.48
IFN γ	70.51	32.72
IL-1b	9.91	4.15
IL-2	12.57	7.18
IL-6	51.83	20.76
IP-10	935.87	518.79
MCP-1	296.35	308.27
MIP-1α	56.65	36.84
MIP-1β	378.87	244.94
RANTES	372.37	2238.82
TNF α	3677.6	1720.55

Figure 3. GM-CSF, IL-6 and TNF secretion



- **Effects on T-cell proliferation**

The immunogenicity assessment of filgrastim was carried out using the Epibase *in vitro* DC:CD4 proliferation assay. Any batch to be determined as significantly immunogenic, should had 2-fold increased response and should had a p-value < 0.05 compared to Blank condition. The results suggest that BP13 was similar to EU-Neupogen in having a low risk for inducing an unwanted CD4+ T-cell responses.

2.4.4. Ecotoxicity/environmental risk assessment

The active substance is a natural substance, the use of which will not alter the concentration or distribution of the substance in the environment. Therefore, filgrastim is not expected to pose a risk to the environment.

2.4.5. Discussion on non-clinical aspects

The comparative *in vitro* data package appears limited to demonstrate the similar functional activity of BP13 and Neupogen. Nevertheless, these studies reflect the principal mode of action of filgrastim, *i.e.* binding to G-CSFR (on BM precursor cells) and initiating cell proliferation. Effects on lineage commitment/differentiation was not included, which is acceptable considering that this is a downstream

effect following the target binding. Assays included the phospho-STAT3 signalling assay demonstrating the downstream pathway of BP13 and Neupogen receptor binding. Nine lots of BP13 and 8 lots of RMP were included to the functional similarity assessment. No information of the lots tested except one (clinical batch) was given under the non-clinical part, but more information is obtainable under the Quality/biosimilarity assessment.

Additional three *in vitro* studies included assessment of immunogenicity. BP13 and Neupogen were similar in their lack of activation of the Toll-like receptors known to provoke innate immune response.

In PBMC activation analysis, two out of three BP13 and Neupogen batches significantly increased GM-CSF and IL-10 secretion, but the increase was similar as well as the responses in other tested cytokines. However, although some variation is expected for cell-based studies, one BP13 batch (R1302-CL-0001) was systematically triggering more secretion of cytokines (GM-CSF, IFN-γ, IL-1β, IL-2, IL-6, IL-10, IP-10, MIP-1α, MIP-1β, RANTES and TNFα) in PBMC than Neupogen and the other two BP13 batches. This effect was considered not explainable solely by two donors (with responses not in line with the observed by the rest of the donors). In the response to the concerns raised, the difference of R1302-CL-0001 batch responses was proposed to be likely attributed to physiological factors and it was pointed out that the values were highly variable within the assay. It was concluded that there was no increased risk of activating an unwanted immune response with BP13 (including batch R1302-CL-0001) and that the potential to induce immunogenicity is comparable to Neupogen batches. The applicant adequately further justified the claim that there is no increased risk of an unwanted immune response triggered by BP13 compared to Neupogen as determined by the cytokine release assay or T-cell proliferation assay (DC:CD4).

There were no other results indicative of differences in the immunogenicity potential, thus, from the totality of evidence point of view and with further clarifications obtained for cytokine and T-cell proliferation assays, BP13 and Neupogen can be considered similar in their immunogenicity potential.

The active substance is a natural substance, the use of which will not alter the concentration or distribution of the substance in the environment. Therefore, filgrastim is not expected to pose a risk to the environment.

2.4.6. Conclusion on the non-clinical aspects

Overall, the primary *in vitro* pharmacodynamic studies provide evidence of similar biological activity between Zefylti and Neupogen.

2.5. Clinical aspects

2.5.1. Introduction

GCP aspects

The clinical trials were performed in accordance with GCP as claimed by the applicant.

The applicant has provided a statement to the effect that clinical trials conducted outside the Community were carried out in accordance with the ethical standards of Directive 2001/20/EC.

- **Tabular overview of clinical studies**

Protocol	Design	Objective(s)	Treatment	Status
BP13-101 (PK/PD similarity)	Phase I, Double-Blind, Randomized, Parallel, Controlled Study to Compare Pharmacokinetics and Pharmacodynamics of BP13 (filgrastim) with EU-approved Neupogen® in Healthy Male Adult Subjects	**Primary** To compare the PK and PD of BP13with EU-approved Neupogen® **Secondary** To compare the PK of BP13 with EU-approved Neupogen® To compare the PD of BP13 (filgrastim) with EU-approved Neupogen® To compare CD34+ cell response between BP13, and EU-approved Neupogen® To explore the potential immunogenicity of BP13 and EU-approved Neupogen® To assess and compare the safety and tolerability of BP13 and EU-approved Neupogen®	Subjects received 5 mcg/kg/day subcutaneous (SC) injection of either BP13 or Neupogen® from Day 1 to Day 5.	Complete

2.5.2. Clinical pharmacology

2.5.2.1. Pharmacokinetics

The pharmacokinetic (PK) similarity of BP13 to Neupogen was investigated in one clinical PK/PD study BP13-101: phase I, single-centre, multiple-dose, randomised, parallel, double-blind, controlled study in healthy adult male subjects. Subjects received 5 µg/kg/day subcutaneous (SC) injection of either BP13 or Neupogen from Day 1 to Day 5 via 1 graduated pre-filled syringe (PFS) on the subject's abdomen, rotating quadrants for each dose.

Venous blood samples for PK were collected on Day 1: Pre-dose (between 5 and 45 minutes prior to dosing), 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 16 and 24h (Day 2); Days 2 to 4: Pre-dose, and Day 5: Pre-dose, 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 16, 24h (Day 6), 36, 48h (Day 7), 60, and 72h (Day 8).

The primary PK parameters were:

- $AUC_{(0-t)}$: Area under the concentration-time curve (AUC) of the drug up to the last quantifiable concentration (starting at Day 5 after study drug administration)
- C_{max}: Maximum observed concentration of the drug in the serum (Day 5).

The secondary PK parameters were:

- $AUC_{(0-24)}$: AUC of the drug from time 0 to 24 hours (Day 1)
- C_{max}: Maximum observed concentration of the drug in the serum (Day 1)
- T_{max}: Time of maximum concentration observed (Day 1 and Day 5)
- $t_{1/2}$: Terminal elimination half-life of the drug (Day 5)
- $AUC_{(0-inf)}$: AUC of the drug extrapolated to infinite time (starting at Day 5 after study drug administration)
- C_{trough}: pre-dose concentration on (Days 2 to 5)

Bioanalytical methods

The bioanalytical methods (developed and validated) used in the clinical study BP13-101 were the following:

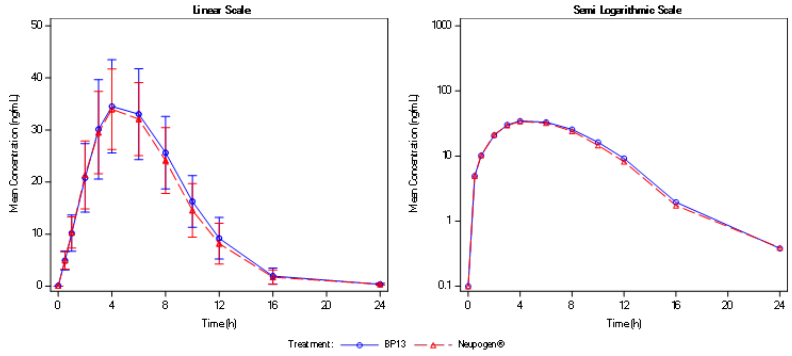
- quantification of filgrastim human serum concentration based on ELISA;
- determination of absolute neutrophil count (ANC) and $CD34^+$ positive cells in human serum performed with flow cytometry;
- immunogenicity assessment including determination of antidrug antibodies (ADA) and neutralizing antibodies (nAb) against filgrastim in human serum. ADA detection utilizing three-tiered approach was done with ligand binding assay based on ECL detection. nAb analysis was based on commercially available genetically modified cells responsive to GCSF and Dual-Glo Luciferase Assay Ready kit.

Results

143 subjects (N = 71 in the BP13 group and N = 72 in the Neupogen group) were included in the PK analysis set.

The arithmetic mean (± SD) serum concentration time data for BP13 and Neupogen in both linear and semilogarithmic scale (PK analysis set) on Day 1 and Day 5 are presented below.

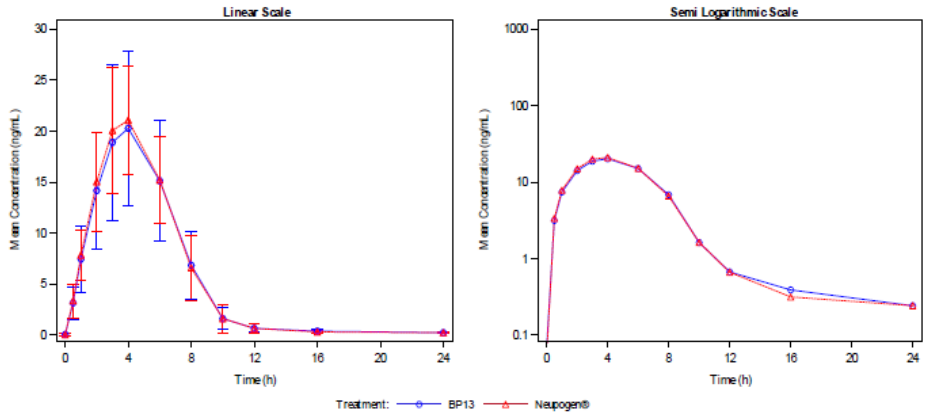
Figure 4. Arithmetic mean (± SD) of serum concentration (ng/ml) time data for BP13 and Neupogen – linear scale and semilogarithmic scale (PK analysis set) – Day 1



Abbreviations: PK = pharmacokinetic; SD = standard deviation

Note: Day 124-hour samples were not presented for three subjects as these samples were collected after dosing on Day 1.

Figure 5. Arithmetic mean (± SD) of serum concentration (ng/ml) time data for BP13 and Neupogen – linear scale and semilogarithmic scale (PK analysis set) – Day 5



Abbreviations: PK = pharmacokinetic; SD = standard deviation

BP13 and Neupogen were biosimilar with respect to the $AUC_{(0-t)}$ and C_{max} (Table 5).

Table 5. Statistical analysis to assess bioequivalence of serum PK parameters: BP13 versus Neupogen at Day 5 (PK analysis set)

	BP13			Neupogen®			Ratio: BP13 / Neupogen®	
	N	GM	90% CI	N	GM	90% CI	GMR	90% CI
$AUC_{(0-t)}$ (h*ng/mL)	71	111.112	(104.908, 117.682)	72	116.025	(109.591, 122.837)	0.958	(0.883, 1.038)
C_{max} (ng/mL)	71	19.481	(18.261, 20.783)	72	20.952	(19.648, 22.342)	0.930	(0.849, 1.019)

N: Number of observations in respective treatments used in the model.
Assessment of bioequivalence was performed using an analysis of variance (ANOVA) including treatment as fixed effect, after logarithmic transformation of the data.
Abbreviations: GM = geometric mean; GMR = geometric mean ratio; CI = confidence interval; C_{max} = maximum observed concentration; AUC = area under the curve.

The inter-individual CV% in the primary PK parameters was moderate with both studied products.

The secondary PK parameters were at similar levels between the test and the reference product groups.

Filgrastim C_{trough} values were low following SC administration 5 µg/kg/day for 5 days and at similar levels between BP13 and Neupogen groups.

2.5.2.2. Pharmacodynamics

Mechanism of action

Filgrastim is a human G-CSF produced by recombinant DNA technology. Endogenous G-CSF is a lineage specific colony-stimulating factor which is produced predominantly by monocytes-macrophages, fibroblasts, and endothelial cells. G-CSF regulates the production of neutrophils within the BM and affects neutrophil progenitor proliferation, differentiation, and selected end-cell functional activation.

Primary and Secondary pharmacology

Pharmacodynamic parameters were evaluated as part of the pivotal PK/PD study BP13-101.

The venous samples for absolute neutrophil count (ANC) were collected on Days 1 to 5 pre-dose, post Day 5 dose at 1, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24h (Day 6), 36, 48h (Day 7), 60, 72h (Day 8), 84, 96h (Day 9), 108 and 120h (Day 10). The venous samples for $CD34^+$ cells were collected between 5 and 45 minutes prior to dosing, Days 1 to 5 pre-dose, post Day 5 dose at 0.5, 1, 2, 4, 6, 8, 10, 12, 16, 24h (Day 6), 36, 48h (Day 7), 60, 72h (Day 8), 84, 96h (Day 9), 108, 120h (Day 10), 144h (Day 11), 168h (Day 12), 216h (Day 14) and 240h (Day 15).

The primary PD endpoints were:

- ANC AUEC(0-t): AUEC from time 0 up to the last scheduled ANC sample (Day 5)
- ANC Emax: Maximum observed ANC (Day 5)

The secondary PD endpoints were:

- Measurement of ANC, $CD34^+$ cell count and Tmax (Day 5)
- $CD34^+$ AUEC(2-t): AUEC of $CD34^+$ cell count from Day 2 through 240 h post-dose on Day 5
- $CD34^+$ Emax: Maximum observed $CD34^+$ cell count on Day 5

The PD analysis set consisted of all subjects who were randomised, received investigational medicinal product (IMP) and completed PD sampling with sufficient PD concentrations to obtain estimates of the

primary PD parameters, and had no major protocol deviations with a relevant impact on PD data. Natural log-transformed AUEC(0-t) and Emax of ANC were analysed using ANOVA. The model included treatment as fixed effect. A comparability range of 90% to 110% was considered for the assessment of bioequivalence. If the back-transformed estimated difference lied between 0.9 and 1.1 then bioequivalence would be concluded.

Results

Altogether 143 subjects were included in the PD analysis set, 71 subjects in the BP13 group and 72 subjects in the Neupogen group.

Primary PD endpoints

Geometric mean (gCV %) of ANC AUEC(0-t) was 1414 h*10^9/L (27.1%) and 1428 h*10^9/L (28.9%) for BP13 and Neupogen, respectively. The GMR (95% CI) for the ratio of BP13:Neupogen for ANC AUEC(0-t) was 0.990 (0.904, 1.084). Geometric mean ANC Emax values were 35.48 10^9/L (22.7%) and 35.41 10^9/L (25.5%) for BP13 and Neupogen, respectively. The GMR (95%CI) for the ratio of BP13:Neupogen for ANC Emax was 1.002 (0.926, 1.084).

Table 6. Statistical analysis of primary PD endpoints (PD analysis set)

	BP13		Neupogen®			Ratio: BP13 / Neupogen®	
	N	GM	95% CI	N	GM	95% CI	GMR [1]	95% CI
ANC $AUEC_{(0-t)}$ ($h*10^9/L$)	71	1413.902	(1325.625, 1508.057)	72	1428.477	(1339.892, 1522.919)	0.990	(0.904, 1.084)
ANC E_{max} ($10^9/L$)	71	35.484	(33.558, 37.521)	72	35.415	(33.505, 37.433)	1.002	(0.926, 1.084)

N: number of observations used in the model.

[1] Assessment of bioequivalence was performed using an analysis of variance (ANOVA) including treatment as fixed effect, after logarithmic transformation of the data.

Abbreviations: GM = geometric mean; GMR = geometric mean ratio; CI = confidence interval; PD = pharmacodynamic; ANC = absolute neutrophil count; AUEC = area under the effect curve; E_{max} = maximum effect.

Secondary endpoints

Geometric mean (gCV %) of $CD34^+$ AUEC(2-t) was 3545 h*cells/µL (64.6%) and 3579 h*cells/µL (57.6%) for BP13 and Neupogen, respectively. Geometric mean (gCV %) of Emax was 58.38 cells/µL (60.4%) and 58.78 cells/µL (51.6%) for BP13 and Neupogen, respectively. Median (min – max) $CD34^+$ Tmax was 11.13 h (0.000 h – 16.03 h) and 12.00 h (3.917 h – 16.02 h) for BP13 and Neupogen, respectively.

2.5.3. Discussion on clinical pharmacology

Bioanalytical methods

The bioanalytical methods used in the clinical study BP13-101 were appropriately described and validated according to the relevant guidelines. Following request, the analysis certificates of critical reagents have been provided for all bioanalytical methods.

Quantification of filgrastim concentration in human serum – BP13 and Neupogen seemed to perform similarly in terms of selectivity, precision, and accuracy. In addition, dilution linearity, hook effect, parallelism and stability (freeze/thaw, short-term freezing, and long-term stability for 398 days at -20 °C) studies were carried out and considered acceptable.

The analysis of clinical samples was reliable within the given accuracy and precision ranges. The reasons for repeat analysis were acceptable and the required criteria for incurred method analysis was met.

Determination of absolute ANC count and $CD34^+$ cells in whole blood – In general, the validation of these methods is considered sufficient.

Detection of ADAs in serum samples – Recombinant mouse anti-human GCSF antibody was used as a positive control and its functionality in the neutralisation of human GSCF antibody was demonstrated.

Screening, confirmatory and tier cut points were determined in healthy serum in acceptable manner. The intra- and inter-assay precisions for screening and confirmation as well as selectivity met the acceptance criteria. The ADA-assay showed high variability in the performance of BP13 and Neupogen in terms of inhibition cut point (34.1% vs. 55.8%), sensitivity (19.7 ng/mL vs. 46.0 ng/mL) and in drug tolerance. For example, at fixed drug level 8 ng/mL the drug tolerance for BP13 was 100 ng/mL and for Neupogen 1500 ng/mL. However, this seems not to be of concern since no ADA positive samples were detected.

The applicant provided the ADA analytical report of serum samples from clinical study BP13-101. The analysis of clinical samples was deemed to be reliable within the given accuracy and precision ranges. Interestingly, almost all clinical samples gave signal comparable to the negative control, and the few positive ADA samples in screening had surprisingly low signal and turned out to be negative in

confirmatory assay. This means that there were no ADA positive samples, which is quite unexpected but has been explained sufficiently by the applicant.

nAb analysis – The screening cut point was determined in acceptable manner and the method showed to be robust and precise. The assay was selective but was affected by haemolysis and lipidaemia at LPC. However, no concerns were raised since no ADA positivity was found in clinical study BP13-101. Otherwise, the method validation followed the current guidance and was considered acceptable.

Pharmacokinetics

The pharmacokinetics of BP13 was investigated in healthy male subjects with a repeated-dose of 5 µg/kg/day SC (clinical study BP13-101). The choice of enrolling healthy male subjects to minimise variability, which may complicate evaluation of PK equivalence, is endorsed. The selected dose is also considered adequate.

The study design (i.e., parallel, multiple-dose study of 5 consecutive daily administrations of either test or reference study product) was according to the recommended for non-pegylated G-CSF on the guideline on similar biological medicinal products containing G-CFS (EMEA/CHMP/BMWP/31329/2005 Rev1). Demographic characteristics were balanced between the treatment groups.

Based on the provided certificates of analysis for the test and the reference product, the batches used in the clinical PK/PD study were appropriate. The protein content of the test product batch was 0.95 mg/ml and of the reference product (sourced from the German market) was 0.96 mg/ml.

The PK sampling time periods can be considered sufficient, although there could have been sampling time-points at 5 and 7 hours after administration of filgrastim to better characterise T_{max} and C_{max}.

The selected primary (i.e., $AUC_{(0\text{-}t)}$ and C_{max} on day 5) and secondary PK parameters (i.e., $AUC_{(0\text{-}24)}$, C_{max}, T_{max} on day 1 and T_{max}, $AUC_{(0\text{-}inf)}$ and $t_{1/2}$ on day 5 and C_{trough} concentrations on days 2 to 5) can be considered adequate.

The statistical methods for demonstrating similarity of average PK are conventional and adequate. Although parallel group study design was used, adjustments for baseline covariates are unnecessary because of the homogeneity of the study population and weight-based dosing of the comparative treatments.

The primary PK parameters ($AUC_{(0\text{-}t)}$ and C_{max} on day 5) with their 90% CIs were within the pre-defined acceptance range of 80-125% (including 100%). BP13 and Neupogen are biosimilar with respect to the extent and rate of absorption of filgrastim. The inter-individual CV% in the primary PK parameters was moderate with both studied products. The secondary PK parameters were at similar levels between the test and the reference product groups.

The mean geometric C_{trough} concentrations on days 2-5 were between 0.25-0.41 ng/ml being at similar level with the test and the reference product. The C_{trough} concentrations were the greatest on day 2 and the lowest on day 5 with both products. There were many subjects whose pre-dose C_{trough} concentrations were BLQ on one or more days, however, the number of subjects with value below vs. above the method of LLOQ was similar across day 1 through day 5.

On day 5, the median $t_{1/2}$ (min, max) for BP13 group and for Neupogen group were reported to be 1.47 h (0.87, 7.47) and 1.42 h (0.78, 6.00), respectively. The range of $t_{1/2}$ (i.e., min, max) was large. The number of timepoints used in the derivation of the $t_{1/2}$ for all subjects was at least 3 (range of 3-7).

For Neupogen, it has been reported that following SC administration, serum concentrations were maintained above 10 ng/ml for 8 to 16 hours. In study BP13-101, after a single SC dose of 5 µg/kg, the mean serum concentration remained > 10 ng/ml up to 12 hours and after multiple doses on day 5 the

mean serum concentrations remained > 10 ng/ml for less than 8 hours. This is reflected in section 5.2 of Zefylti SmPC.

No clinical studies in target population and special population as well as no interaction studies were conducted, as such studies are not considered needed.

Pharmacodynamics

The pharmacodynamics of BP13 was investigated as part of the PK/PD study BP13-101 in healthy male subjects. From the PD perspective, the study design, i.e., a multiple-dose study consisting of administration of 5 µg/kg/day SC injection for 5 days, as well as the primary PD endpoints, $AUEC_{(0-t)}$ and ANC E_{max}, determined after the last dose (day 5), are in line with the draft guideline (EMEA/CHMP/BMWP/31329/2005 Rev 1) and acceptable. The comparability limits were within the requirements of the draft guideline, which states that a predefined comparability range of 90-111% would be acceptable without further justification.

The statistical methods for demonstrating similarity of average PD are conventional and adequate. Although parallel group study design was used, adjustments for baseline covariates are considered unnecessary because of the homogeneity of the study population and weight-based dosing of the comparative treatments.

In terms of the primary PD endpoints, the geometric mean ratio (95% CI) was 0.990 (0.904, 1.084) for ANC AUEC(0-t) and 1.002 (0.926, 1.084) for ANC Emax. Due to missing ANC values, the applicant also reproduced the ANC analyses, setting the missing values as missing (instead of zero, as in the primary analysis). As the 95% CI's were within the acceptance range of 90–111% (EMEA/CHMP/BMWP/31329/2005 Rev 1) both in the primary analysis and reproduced analysis, BP13 and Neupogen could be concluded to be biosimilar in terms of PD.

Overall, the secondary endpoints $CD34^+$ AUEC (2-t), $CD34^+$ Emax and T max for ANC and $CD34^+$ cells appeared similar between BP13 and Neupogen. On request, the applicant performed statistical analysis for $CD34^+$ AUEC(0-t) and $CD34^+$ Emax, which were not pre-planned. Geometric mean (gCV%) of $CD34^+$ AUEC(0-t) was 2580 h*cells/µL (62.2%) and 2606 h*cells/µL (56.1%) for BP13 and Neupogen, respectively. The GMR (95% CI) for the ratio of BP13:Neupogen was 0.990 (0.808, 1.212) for $CD34^+$ AUEC(0-t) and 0.993 (0.819, 1.205) for $CD34^+$ Emax. Although the 95% CIs for these PD endpoints fall out of the 0.9–1.11 range, the GMRs are close to 1 supporting the overall conclusion of biosimilarity in terms of PD.

2.5.4. Conclusions on clinical pharmacology

The available PK/PD data support biosimilarity of BP13 versus the EU reference product.

2.5.5. Clinical efficacy

No clinical efficacy studies were conducted/submitted by the applicant.

2.5.6. Clinical safety

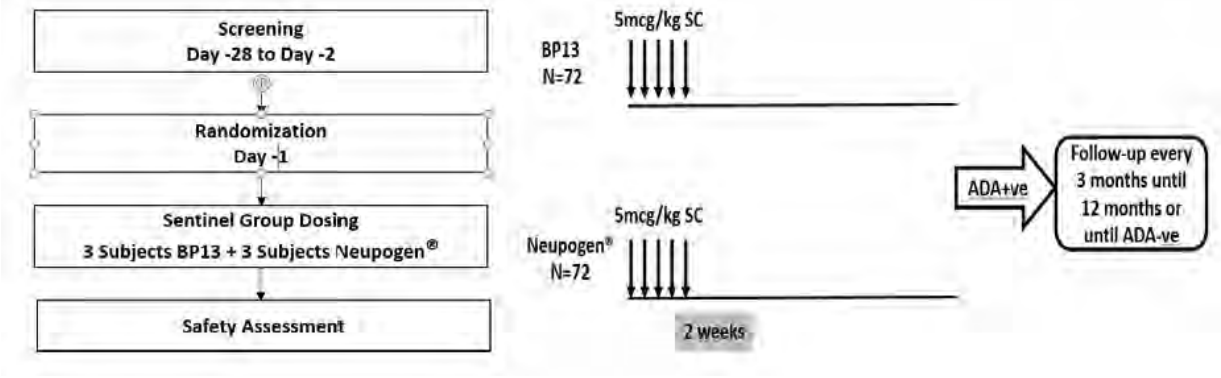
The BP13 development program consisted of one Phase 1 study (BP13-101) in healthy male adult subjects. Study BP13-101 was a single-centre, double-blind, randomised, parallel, controlled study to compare the PK and PD of BP13 with the RMP Neupogen. Comparative safety, tolerability and immunogenicity were secondary objectives of the study.

With reference to the safety assessment, a complete physical examination was included, and at a minimum, assessments of the cardiovascular, respiratory, gastrointestinal, and neurological systems. Height and weight were also measured and recorded. Temperature, pulse rate, electrocardiogram (ECG) and blood pressure were assessed. A splenic ultrasound was to be carried out to rule out any splenic abnormalities before the subject was dosed with IMPs. Haematology, clinical chemistry, coagulation, iron profile, and urinalysis were also assessed as safety evaluation. Injection sites were assessed for reactions prior to each injection and at other times specified in the schedule of assessments.

A total of 146 healthy male subjects were randomised in a 1:1 ratio to one of the treatment arms and received either BP13 (N=72) or Neupogen (N=72) (two subjects were randomised but did not receive the study treatment). Subjects received 5 mcg/kg/day subcutaneous (SC) injection of either BP13 or Neupogen from Day 1 to Day 5. A first group of 6 sentinel subjects (3 subjects receiving BP13 and 3 subjects receiving Neupogen) were dosed first to establish the safety profile (e.g., AEs, TEAEs, SAEs, onset of serious allergic reactions, including anaphylaxis) prior to dosing the rest of the study population. The test product or the reference product was administered subcutaneously via 1 graduated PFS on the subject's abdomen. The study comprised of a screening period (Day -28 to Day -2), an inpatient period (Day -1 to Day 10) when the subject received IMP on Days 1 to 5 and a follow-up/return visits on Day 11, Day 12, Day 14, and Day 15.

The total study duration for each subject was approximately 15 days (excluding the 28-day screening period). If a subject tested positive for anti-drug antibodies (ADA), was to be followed every 3 months until 12 months or until tested negative for ADA.

Figure 6. Study design and plan



Abbreviations: ADA=Anti-drug antibodies; SC=Subcutaneous.

Demographics and other Characteristics of Study Population

Subjects were between the ages of 18 and 52 years (median 27.0 years). Most subjects were White (116/144 [80.6%] subjects) and were Not Hispanic or Latino (105/144 [72.9%] subjects). Subject characteristics, including height, weight, and BMI, were generally well balanced between the treatment arms.

2.5.6.1. Patient exposure

Table 7. Summary of treatment exposure and compliance (safety analysis set)

Categories	BP13 (N=72)	Neupogen (N=72)	Overall (N=144)
Total Dose received (mcg)			
n	72	72	144
Mean	1893.72	1905	1899.36
SD	259.497	213.951	237.051
Median	1878.75	1880	1878.75
Min, Max	798.0,2345.0	1365.0,2350.0	798.0,2350.0
Duration of Exposure (days)			
n	72	72	144
Mean	4.96	5	4.98
SD	0.354	0	0.25
Median	5	5	5
Min, Max	2.0, 5.0	5.0, 5.0	2.0, 5.0
Compliance (%)			
n	72	72	144
Mean	99.17	100	99.58
SD	7.071	0	5
Median	100	100	100
Min, Max	40.0, 100.0	100.0, 100.0	40.0, 100.0

Abbreviations: Max = maximum; Min = minimum; SD = standard deviation. Percentages are calculated based on the number of patients in the respective treatment group under Safety analysis set. Duration of exposure is defined as the duration of time from the start of BP13 or Neupogen administration to the stop of administration. The overall drug compliance is defined as percentage of total dose administered in mg during the study divided by the expected total dose in mg.

Overall, 144/146 (98.6%) of the randomised subjects were included in Safety Analysis Set and 143/146 (97.9%) subjects were included in the PK and PD analysis sets.

Adherence to the study was good. Discontinuations were overall rare; only one person discontinued due to AEs. This was a mild, Grade 1 case of urticaria, which was treated with cetirizine and subsequently resolved.

The detailed description of the design and conduct of study BP13-101 and the key baseline patient and disease characteristics are found in the PK/PD section.

Table 8. Summary of subject disposition (Screened Analysis Set)

Categories	BP13 (N=74) n (%)	Neupogen® (N=72) n (%)	Overall (N=344) n (%)
Screened			344
Screen failure [1]			198 (57.6)
Reason for screen failure [1]			
Inclusion/exclusion criteria not met [1]			123 (35.8)
Other			75 (21.8)
Randomized [2]	74 (100.0)	72 (100.0)	146 (100.0)
Subjects dosed [2]	72 (97.3)	72 (100.0)	144 (98.6)
Completed [2]	67 (90.5)	68 (94.4)	135 (92.5)
Discontinued Subjects	7 (9.5)	4 (5.6)	11 (7.5)
Reason for discontinuation from study [2]			
Adverse event	1 (1.4)	0	1 (0.7)
Physician decision	1 (1.4)	0	1 (0.7)
Consent withdrawal by subject	2 (2.7)	2 (2.8)	4 (2.7)
Other	3 (4.1)	2 (2.8)	5 (3.4)

n: The number of subjects in the Randomised Analysis Set [1] Percentage calculated using the number of subjects in Screened Analysis Set, as denominator (n/N*100). [2] Percentage calculated using the number of subjects randomised for each treatment group/overall, as denominator (n/N*100). Note: Two subjects were randomised but did not receive the study treatment and discontinued due to Consent withdrawal by subject and Physician decision respectively.

2.5.6.2. Adverse events

A total of 253 adverse events (AEs), out of which 246 treatment-emergent adverse events (TEAEs), were reported in 119/144 (82.6%) subjects; 129 AEs were reported in 62/72 (86.1%) subjects in the BP13 arm, and 124 AEs were reported in 57/72 (79.2%) subjects in the Neupogen arm. There were no major differences in the number of TEAEs reported between treatment arms.

Most TEAEs were considered to be mild (240 events in 119/144 [82.6%] subjects overall; 122 events in 62/72 [86.1%] subjects in the BP13 arm and 118 events in 57/72 [79.2%] subjects in the Neupogen arm) and Grade 1 in severity (239 events in 119/144 [82.6%] subjects overall; 121 events in 62/72 [86.1%] subjects in the BP13 arm and 118 events in 57/72 [79.2%] subjects in the Neupogen arm). No TEAEs of Grade 3, 4 and 5 severity or severe intensity were reported during the study.

No serious TEAEs or deaths were reported during the study in either treatment arm.

A total of 46 events in 42/144 (29.2%) subjects were considered to be probably related to the study drug (25 events in 22/72 [30.6%] subjects in the BP13 arm and 21 events in 20/72 [27.8%] subjects in the Neupogen arm) and a total of 125 events in 87/144 (60.4%) subjects were considered to be possibly related to the study drug (66 events in 46/72 [63.9%] subjects in the BP13 arm and 59 events in 41/72 [56.9%] subjects in the Neupogen arm).

A total of 36 events in 29/144 (20.1%) subjects were considered to be unlikely related to the study drug (18 events in 15/72 [20.8%] subjects in the BP13 arm and 18 events in 14/72 [19.4%] subjects in the Neupogen arm) and 46 events in 38/144 (26.4%) subjects were considered to be not related to the study drug (20 events in 18/72 [25.0%] subjects in the BP13 arm and 26 events in 20/72 (27.8%) subjects in the Neupogen arm).

No action (dose not changed) was taken with the IMP due to TEAEs in the majority of subjects (183 events in 108/144 [75.0%] subjects; 95 events in 56/72 [77.8%] subjects in the BP13 arm and 88 events in 52/72 [72.2%] subjects in the Neupogen arm). Action taken with the study drug was "not applicable" for 69 events in 49/144 (34%) of subjects (33 events in 25/72 [34.7%] subjects in the BP13 arm and 36 events in 24/72 [33.3%] subjects in the Neupogen arm). BP13 was permanently withdrawn due a TEAE in one subject.

A total of 247 events in 117/144 (81.3%) subjects (127 events in 62/72 [86.1%] subjects in the BP13 arm and 120 events in 55/72 [76.4%] subjects in the Neupogen arm) had resolved by the end of the study. Overall, 5 events in 5/144 (3.5%) subjects (1 event in 1/72 [1.4%] subjects in the BP13 arm and 4 events in 4/72 [5.6%] subjects in the Neupogen arm) had not resolved by the end of the study.

Table 9. Overview of adverse events (safety analysis set)

Category	BP13 (N=72) n (%) E	Neupogen (N=72) n (%) E	Overall (N=144) (N=72) n (%) E
Adverse events	62 (86.1) 129	57 (79.2) 124	119 (82.6) 253
TEAEs	62 (86.1) 126	57 (79.2) 120	119 (82.6) 246
Intensity/Severity			
Mild	62 (86.1) 122	57 (79.2) 118	119 (82.6) 240
Moderate	4 (5.6) 4	2 (2.8) 2	6 (4.2) 6
Severe	0	0	0
CTCAE Toxicity grade			
Grade 1: Mild	62 (86.1) 121	57 (79.2) 118	119 (82.6) 239
Grade 2: Moderate	5 (6.9) 5	2 (2.8) 2	7 (4.9) 7
Grade 3: Severe or medically significant	0	0	0
Grade 4: Life-threatening or disabling	0	0	0
Grade 5: Death related to AE	0	0	0
Serious TEAEs			
Yes	0	0	0
No	62 (86.1) 126	57 (79.2) 120	119 (82.6) 246
Relationship to study treatment			
Probably related	22 (30.6) 25	20 (27.8) 21	42 (29.2) 46
Possibly related	46 (63.9) 66	41 (56.9) 59	87 (60.4) 125
Unlikely related	15 (20.8) 18	14 (19.4) 18	29 (20.1) 36
Not related	16 (22.2) 17	18 (25) 22	34 (23.6) 39
Action taken with study treatment			
Dose not changed	56 (77.8) 95	52 (72.2) 88	108 (75.0) 183

Drug interrupted	0	0	0
Drug withdrawn	1 (1.4) 1	0	1 (0.7) 1
Unknown	0	0	0
Not applicable	25 (34.7) 33	24 (33.3) 36	49 (34.0) 69
Outcome			
Fatal	0	0	0
Not recovered or not resolved	1 (1.4) 1	4 (5.6) 4	5 (3.5) 5
Recovered or resolved	62 (86.1) 127	55 (76.4) 120	117 (81.3) 247
Recovered or resolved with sequelae	0	0	0
Recovering or resolving	0	0	0
Unknown	1 (1.4) 1	0	1 (0.7) 1
Other	0	0	0

n: number of subjects reporting at least one AE in each category; N: The number of subjects in the Safety Analysis Set; E = number of events. Percentages were calculated using the number of subjects in the Safety Analysis Set as the denominator (n/N*100). All TAEs were coded using MedDRA version 24.0. TEAEs include any AEs occurring or worsening after the first dose of study medication. Abbreviations: AE = adverse event; CTCAE = Common Terminology Criteria for Adverse Events; MedDRA = Medical Dictionary for Regulatory Activities; TEAE: treatment-emergent adverse event.

TEAEs were summarised by system organ class (SOC) and preferred term (PT), CTCAE grade severity, severity, relationship with IMP, action taken with study drug, and by outcome.

Table 10. Summary of treatment-emergent adverse events by system organ class and preferred term (safety analysis set)

System Organ Class/Preferred Term	BP13 (N=72) n (%)	Neupogen (N=72) n (%)	Overall (N=144) n (%)
Blood and lymphatic system disorders	0	1 (1.4) 1	1 (0.7) 1
Lymphadenopathy	0	1 (1.4) 1	1 (0.7) 1
Cardiac disorders	1 (1.4) 1	0	1 (0.7) 1
Sinus tachycardia	1 (1.4) 1	0	1 (0.7) 1
Ear and labyrinth disorders	1 (1.4) 1	0	1 (0.7) 1
Hypoacusis	1 (1.4) 1	0	1 (0.7) 1
Eye disorders	1 (1.4) 1	1 (1.4) 1	2 (1.4) 2
Dacryostenosis acquired	1 (1.4) 1	0	1 (0.7) 1
Eyelid irritation	0	1 (1.4) 1	1 (0.7) 1
Gastrointestinal disorders	7 (9.7) 8	9 (12.5) 10	16 (11.1) 18
Abdominal discomfort	1 (1.4) 1	0	1 (0.7) 1
Abdominal pain	2 (2.8) 2	2 (2.8) 3	4 (2.8) 5
Abdominal pain upper	1 (1.4) 1	2 (2.8) 2	3 (2.1) 3
Diarrhoea	1 (1.4) 1	1 (1.4) 1	2 (1.4) 2
Dry mouth	0	1 (1.4) 1	1 (0.7) 1
Intra-abdominal haematoma	1 (1.4) 1	0	1 (0.7) 1
Nausea	2 (2.8) 2	2 (2.8) 2	4 (2.8) 4
Rectal haemorrhage	0	1 (1.4) 1	1 (0.7) 1
General disorders and administration site conditions	16 (22.2) 18	17 (23.6) 17	33 (22.9) 35

Catheter site bruise	0	1 (1.4) 1	1 (0.7) 1
Catheter site erythema	0	1 (1.4) 1	1 (0.7) 1
Catheter site haematoma	0	1 (1.4) 1	1 (0.7) 1
Catheter site pain	7 (9.7) 7	8 (11.1) 8	15 (10.4) 15
Catheter site related reaction	0	1 (1.4) 1	1 (0.7) 1
Chills	1 (1.4) 1	0	1 (0.7) 1
Fatigue	2 (2.8) 2	2 (2.8) 2	4 (2.8) 4
Infusion site thrombosis	1 (1.4) 1	0	1 (0.7) 1
Injection site erythema	2 (2.8) 2	0	2 (1.4) 2
Injection site pain	1 (1.4) 1	0	1 (0.7) 1
Injection site pruritus	1 (1.4) 1	0	1 (0.7) 1
Malaise	2 (2.8) 2	1 (1.4) 1	3 (2.1) 3
Non-cardiac chest pain	0	1 (1.4) 1	1 (0.7) 1
Vessel puncture site bruise	0	1 (1.4) 1	1 (0.7) 1
Vessel puncture site haematoma	1 (1.4) 1	0	1 (0.7) 1
Infections and infestations	2 (2.8) 2	1 (1.4) 1	3 (2.1) 3
Cellulitis	1 (1.4) 1	0	1 (0.7) 1
Ear infection	1 (1.4) 1	0	1 (0.7) 1
Upper respiratory tract infection	0	1 (1.4) 1	1 (0.7) 1
Injury, poisoning and procedural complications	4 (5.6) 4	1 (1.4) 1	5 (3.5) 5
Contusion	1 (1.4) 1	1 (1.4) 1	2 (1.4) 2
Joint injury	1 (1.4) 1	0	1 (0.7) 1
Skin abrasion	1 (1.4) 1	0	1 (0.7) 1
Thermal burn	1 (1.4) 1	0	1 (0.7) 1
Musculoskeletal and connective tissue disorders	47 (65.3) 52	44 (61.1) 50	91 (63.2) 102
Arthralgia	2 (2.8) 2	2 (2.8) 2	4 (2.8) 4
Back pain	24 (33.3) 24	19 (26.4) 19	43 (29.9) 43
Bone pain	18 (25.0) 18	21 (29.2) 21	39 (27.1) 39
Musculoskeletal pain	1 (1.4) 1	5 (6.9) 5	6 (4.2) 6
Musculoskeletal stiffness	1 (1.4) 1	0	1 (0.7) 1
Myalgia	3 (4.2) 3	1 (1.4) 1	4 (2.8) 4
Pain in extremity	3 (4.2) 3	1 (1.4) 1	4 (2.8) 4
Tendonitis	0	1 (1.4) 1	1 (0.7) 1
Nervous system disorders	26 (36.1) 27	26 (36.1) 31	52 (36.1) 58
Dizziness	1 (1.4) 1	0	1 (0.7) 1
Dysgeusia	0	2 (2.8) 2	2 (1.4) 2
Headache	24 (33.3) 25	23 (31.9) 25	47 (32.6) 50
Lethargy	0	1 (1.4) 1	1 (0.7) 1
Paraesthesia	0	1 (1.4) 1	1 (0.7) 1
Presyncope	1 (1.4) 1	1 (1.4) 2	2 (1.4) 3
Psychiatric disorders	0	1 (1.4) 1	1 (0.7) 1
Anxiety	0	1 (1.4) 1	1 (0.7) 1
Respiratory, thoracic and mediastinal disorders	5 (6.9) 5	1 (1.4) 1	6 (4.2) 6
Dyspnoea	0	1 (1.4) 1	1 (0.7) 1
Nasal congestion	1 (1.4) 1	0	1 (0.7) 1
Oropharyngeal discomfort	1 (1.4) 1	0	1 (0.7) 1
Oropharyngeal pain	2 (2.8) 2	0	2 (1.4) 2
Rhinorrhoea	1 (1.4) 1	0	1 (0.7) 1
Skin and subcutaneous tissue disorders	2 (2.8) 2	5 (6.9) 5	7 (4.9) 7
Acne	0	1 (1.4) 1	1 (0.7) 1

Dry skin	1 (1.4) 1	1 (1.4) 1	2 (1.4) 2
Erythema	0	2 (2.8) 2	2 (1.4) 2
Rash	0	1 (1.4) 1	1 (0.7) 1
Urticaria	1 (1.4) 1	0	1 (0.7) 1
Vascular disorders	5 (6.9) 5	1 (1.4) 1	6 (4.2) 6
Flushing	1 (1.4) 1	0	1 (0.7) 1
Haematoma	1 (1.4) 1	1 (1.4) 1	2 (1.4) 2
Orthostatic hypotension	1 (1.4) 1	0	1 (0.7) 1
Thrombophlebitis	2 (2.8) 2	0	2 (1.4) 2

n: number of subjects reporting at least one AE in each category; N: The number of subjects in the Safety Analysis Set; E: Number of events. Percentages were calculated using the number of subjects in the Safety Analysis Set as the denominator (n/N*100). All AEs were coded using MedDRA version 24.0. Treatment-emergent adverse events (TEAEs) include any AEs occurring or worsening on or after the first dose of study medication. Abbreviations: AE = adverse event; MedDRA = Medical Dictionary for Regulatory Activities

Analysis of adverse events

The TEAEs that were reported per SOC included:

- Musculoskeletal and connective tissue disorders (102 events in 91/144 [63.2%] subjects in the overall group; 52 events in 47/72 [65.3%] subjects in the BP13 arm and 50 events in 44/72 [61.1%] subjects in the Neupogen arm). Overall, 43 events of back pain in 43/144 (29.9%) subjects were reported (24 events in 24/72 [33.3%] subjects in the BP13 arm and 19 events in 19/72 [26.4%] subjects in the Neupogen arm). Overall, 39 events of bone pain in 39/144 (27.1%) subjects were reported (18 events in 18/72 [25.0%] subjects in the BP13 arm and 21 events in 21/72 [29.2%] subjects in the Neupogen arm).
- Nervous system disorders (58 events in 52/144 [36.1%] subjects in the overall group; 27 events in 26/72 [36.1%] subjects in the BP13 Neupogen arm and 31 events in 26/72 [36.1%] subjects in the Neupogen arm). Overall, 50 events of headache in 47/144 (32.6%) subjects were reported (25 events in 24/72 [33.3%] subjects in the BP13 arm and 25 events in 23/72 [31.9%] subjects in the Neupogen arm).
- General disorders and administration site conditions (35 events in 33/144 [22.9%] subjects in the overall group; 18 events in 16/72 [22.2%] subjects in the BP13 arm and 17 events in 17/72 [23.6%] subjects in the Neupogen arm). Overall, 15 events of catheter site pain in 15/144 (10.4%) subjects were reported (7 events in 7/72 [9.7%] subjects in the BP13 arm and 8 events in 8/72 [11.1%] subjects in the Neupogen arm).
- Gastrointestinal disorders (18 events in 16/144 [11.1%] subjects in the overall group; 8 events in 7/72 [9.7%] subjects in the BP13 arm and 10 events in 9/72 [12.5%] subjects in the Neupogen arm).

Toxicity and severity of AEs

Overall, a total of 246 events in 119/144 (82.6%) subjects were of Grade 1 and mild in severity. Seven TEAEs in 7/144 (4.9%) subjects were assessed to be of Grade 2 severity, which included one event each of cellulitis, injection site erythema, ear infection, thrombophlebitis, and abdominal pain upper in subjects in the BP13 arm, and rectal haemorrhage and musculoskeletal pain in subjects in the Neupogen arm.

Six TEAEs in 6/144 (4.2%) subjects were of moderate severity which included one event each of cellulitis, ear infection, thrombophlebitis, and abdominal pain upper in subjects in the BP13 arm, and rectal haemorrhage and musculoskeletal pain in subjects in the Neupogen arm.

Potential relationship of adverse events to study treatment

Among the TEAEs that were probably or possibly related to IMP, the most frequently reported TEAEs (reported in ≥ 5% of overall subjects) included:

- Back pain: 40 events in 40/144 (27.8%) subjects (22 events in 22/72 [30.6%] subjects in the BP13 arm and 18 events in 18/72 [25.0%] subjects in the Neupogen arm) were considered possibly related.
- Bone pain: 38 events in 38/144 (26.4%) subjects (18 events in 18/72 [25.0%] subjects in the BP13 arm and 20 events in 20/72 [27.8%] subjects in the Neupogen arm) were considered probably related.
- Headache: 45 events in 43/144 (29.9%) subjects (23 events in 22/72 [30.6%] subjects in the BP13 arm and 22 events in 21/72 [29.2%] subjects in the Neupogen arm) were considered possibly related.

While the incidence of IMP-related back pain was marginally higher in subjects in the BP13 arm when compared to subjects in the Neupogen arm, there was no major imbalance in incidence of other IMP-related TEAEs.

2.5.6.3. Serious adverse event/deaths/other significant events

No deaths or SAEs was reported during the BP13-101 study in either treatment arm.

Other Safety Findings (AEs of special interest, AESI)

Local injection site reactions (ISRs)

A total of 5 TEAEs of ISRs (infusion site thrombosis, injection site erythema, injection site pain, and injection site pruritus) in 5 subjects were reported; all were mild and reported in subjects in the BP13 arm and none in subjects in the Neupogen arm.

Calculation of risk ratios for bone pain events, myalgia events

Bone pain was reported in 18/72 (25%) subjects in the BP13 arm and in 21/72 (29.2%) subjects in the Neupogen arm. Myalgia was reported in 3/72 (4.2%) subjects in the BP13 arm and in 1/72 (1.4%) subjects in the Neupogen arm. The subjects in the BP13 arm had 0.86 times the risk of bone pain events and 3 times the risk of myalgia events compared the subjects in the Neupogen arm.

Table 11. Summary of risk ratio of bone pain events and myalgia events (safety analysis set)

Event	BP13 (N=72) n (%)	Neupogen® (N=72) n (%)	Risk Ratio (95 % CI)
Bone Pain			
Yes	18 (25.0)	21 (29.2)	0.86 (0.500, 1.468)
No	54 (75.0)	51 (70.8)	
Myalgia			
Yes	3 (4.2)	1 (1.4)	3.00 (0.320, 28.165)
No	69 (95.8)	71 (98.6)	

n: Number of subjects reporting at least one event in each category. N: The number of subjects in the Safety Analysis Set. Percentages were calculated using the number of subjects in the Safety Analysis Set as the denominator (n/N*100).

2.5.6.4. Laboratory findings

According to the applicant, no relevant trends were identified in the investigated clinical laboratory parameters, vital signs, or ECG results and none of the abnormal results reported for these evaluations were considered clinically significant.

2.5.6.5. In vitro biomarker test for patient selection for safety

Not applicable.

2.5.6.6. Safety in special populations

Not applicable.

2.5.6.7. Immunological events

Blood samples of 5 mL were collected for measurement of ADAs as specified in the Schedule of activities (SoA). Each whole blood sample was processed for serum. Antibodies to filgrastim, filgrastim-GCSF, and GCSF were evaluated in serum samples. Additionally, serum samples were also collected at the final visit from subjects who discontinued IMPs or were withdrawn from the study.

Serum samples were screened for antibodies binding to the IMP and the titre of confirmed positive samples were reported. Other analyses could be performed to verify the stability of antibodies to the IMP and/or further characterise the immunogenicity of the IMP.

The detection and characterisation of antibodies to filgrastim were performed using a validated assay method by or under the supervision of the Sponsor. All samples collected for detection of antibodies to the IMP were evaluated for IMPs serum concentration to enable interpretation of the antibody data. Antibodies were further characterised and/or evaluated for their ability to neutralise the activity of the IMP.

Any ADA-positive subject was followed up every 3 months until 12 months or until the subject was ADA-negative, whichever came first.

Anti-drug antibodies and neutralizing antibodies

According to the applicant none of the subjects in BP13 arm and Neupogen arm were confirmed to be ADA positive at any time-point of the study. It is acknowledged that antibodies against rG-CSF have been reported to develop infrequently and have previously not been associated with relevant consequences for efficacy or safety. Despite this, the complete lack of ADA response is somewhat unexpected. The applicant sufficiently discussed this finding. For assessment of method validation, including reliability of the results, see Clinical pharmacology / Bioanalytical methods.

2.5.6.8. Safety related to drug-drug interactions and other interactions

Not applicable.

2.5.6.9. Discontinuation due to adverse events

In study BP13-101, 11/146 (7.5%) subjects discontinued the study due to the following reasons:

- 1/146 (0.7%) due to Physician's decision and AE (1 subject each, both in the BP13 arm).
- 4/146 (2.7%) due to withdrawal of consent (2 subjects in each arm), and 5/146 (3.4%) due to other reason (travel to site due to geographic distance, death in family, work commitments, other commitments, and refusal to attend outpatient visits) (3 subjects in the BP13 arm and 2 subjects in the Neupogen arm).

Discontinuations were overall rare and only one was ascribed to TEAEs (in the BP13 arm). This was a case of mild, Grade 1 urticaria, possibly related to BP13. Although the AE was not considered serious, the IMP was withdrawn and the subject discontinued the study, as it was judged that repeat exposure could have precipitated a more significant reaction. The subject received treatment with cetirizine and the urticaria resolved. The observations did not provide any new safety findings or concerns in association with BP13.

2.5.6.10. Post marketing experience

Not applicable.

2.5.7. Discussion on clinical safety

The safety assessment of BP13 is based on the Phase I study BP13-101, including 144 healthy male adults. A secondary objective of this clinical study was to compare safety between the biosimilar BP13 and the reference product Neupogen. This is in accordance with requirements laid out in the draft Guideline on similar biological medicinal products containing recombinant granulocyte-colony stimulating factor (rG-CSF) EMEA/CHMP/BMWP/31329/2005 Rev 1. While the previous version of this guideline requested a comparative clinical trial in most cases, the revised guideline focusses on demonstration of biosimilarity based on a strong and convincing physicochemical and functional data package and comparable pharmacokinetic and pharmacodynamic profiles. No long-term data beyond the 15 days duration of the study were accrued, which in this setting is considered acceptable.

Overall, the provided safety database could be considered sufficient for establishing the safety of BP13 considering the well-known safety profile of this active substance and its nature, i.e., a biosimilar.

Exposure

A total of 344 subjects were screened for the study BP13-101 and 146 subjects were randomised, with 74 subjects randomised to BP13 and 72 subjects randomised to Neupogen. Overall, 144/146 (98.6%) of the randomised subjects were included in Safety Analysis Set, 72 subjects dosed in the BP13 arm and 72 subjects dosed in the Neupogen arm. In all, 135/146 (92.5%) subjects completed treatment, 67/74 (90.5%) subjects in the BP13 arm and 68/72 (94.4%) subjects in the Neupogen arm.

Overall, 11/146 (7.5%) subjects discontinued the study due to the following reasons: 1/146 (0.7%) due to Physician's decision and AE (1 subject each, both in BP13 arm), 4/146 (2.7%) due to withdrawal of consent (2 subjects in each arm), and 5/146 (3.4%) due to other reason (travel to site due to geographic distance, death in family, work commitments, other commitments and refusal to attend outpatient visits) (3 subjects in the BP13 arm and 2 subjects in the Neupogen arm).

The total study duration for each subject was approximately 15 days (excluding the 28-day screening period). No data beyond this time period were accrued, which, as per guidance, is considered acceptable.

Safety results in study BP13-101

A total of 253 AEs, out of which 246 TEAEs, were reported in 119/144 (82.6%) subjects; 129 AEs were reported in 62/72 (86.1%) subjects in the BP13 arm, and 124 AEs were reported in 57/72 (79.2%) subjects in Neupogen arm. There were no major differences in the number of TEAEs reported between the treatment arms.

Most commonly reported PTs were headache, back pain, bone pain and catheter site pain. All other AEs were mainly single cases. The reported AEs were generally balanced between the treatment groups (see also AESI below).

Most TEAEs were considered to be mild (240 events in 119/144 [82.6%] subjects overall; 122 events in 62/72 [86.1%] subjects in the BP13 arm and 118 events in 57/72 [79.2%] subjects in the Neupogen arm) and Grade 1, as per CTCAE grading, in severity (239 events in 119/144 [82.6%] subjects overall; 121 events in 62/72 [86.1%] subjects in the BP13 arm and 118 events in 57/72 [79.2%] subjects in the Neupogen arm). No TEAEs of Grade 3, 4 and 5 severity or of severe intensity were reported during the study.

A total of 46 events in 42/144 (29.2%) subjects were considered to be probably related to the study drug (25 events in 22/72 [30.6%] subjects in the BP13 arm and 21 events in 20/72 [27.8%] subjects in the Neupogen arm) and a total of 125 events in 87/144 (60.4%) subjects were considered to be possibly related to the study drug (66 events in 46/72 [63.9%] subjects in the BP13 arm and 59 events in 41/72 [56.9%] subjects in the Neupogen arm). While the incidence of IMP-related back pain was marginally higher in subjects in the BP13 arm when compared to subjects in the Neupogen arm, there was no major imbalance in incidence of other IMP-related TEAEs.

A total of 36 events in 29/144 (20.1%) subjects were considered to be unlikely related to study drug (18 events in 15/72 [20.8%] subjects in the BP13 arm and 18 events in 14/72 [19.4%] subjects in the Neupogen arm) and 46 events in 38/144 (26.4%) subjects were considered to be not related to study drug (20 events in 18/72 [25.0%] subjects in the BP13 arm and 26 events in 20/72 (27.8%) subjects in the Neupogen arm).

No action (dose not changed) was taken with the IMP due to TEAEs in the majority of subjects (183 events in 108/144 [75.0%] subjects; 95 events in 56/72 [77.8%] subjects in the BP13 arm and 88 events in 52/72 [72.2%] subjects in the Neupogen arm). Action taken with the study drug was "not applicable" for 69 events in 49/144 (34%) of subjects (33 events in 25/72 [34.7%] subjects in the BP13 arm and 36 events in 24/72 [33.3%] subjects in the Neupogen arm. BP13 was permanently withdrawn due to a TEAE in one subject.

Thus, in the context of the AEs reported in the study BP13-101 no new or unexpected safety finding were clearly evident.

Deaths and SAEs

No deaths or serious TEAEs were reported in the study.

Laboratory results

No relevant trends were identified in the investigated clinical laboratory parameters, vital signs, or ECG results and none of the abnormal results reported for these evaluations were considered clinically significant. Overall, it can be agreed with the applicant that the changes in the laboratory results were in general modest in size and within expected physiological variation between determinations. The few, larger changes from baseline observed were mainly single occurrences, evenly distributed between the treatment groups and were not reflective of progressive deviations or changes.

AEs of special interest, AESI

A total of 5 TEAEs of ISRs (infusion site thrombosis, injection site erythema, injection site pain, and injection site pruritus) in 5 subjects were reported; all mild and reported in subjects in the BP13 arm and none in subjects in the Neupogen arm. Based on the data provided, there appeared to be no formulation or device attributes that could readily explain this sight discrepancy in numbers between the treatment groups. The extent and type of ISRs observed are in line with previously reported events in other similar medicinal products. Thus, this observation is considered not to imply any additional safety concern with BP13 treatment.

Immunogenicity

As with all therapeutic proteins, there is a potential for BP13 for immunogenicity. Rate of generation of antibodies against filgrastim has generally been low. However, in this study none of the subjects in BP13 arm and Neupogen arm were confirmed to be ADA positive at any time-point of the study. This was somewhat unexpected and thus, on request, the applicant adequately clarified this issue (see also Bioanalytical methods). No apparent reason for this difference between EU-Neupogen and BP13 with respect to ADA responses was identified.

Subgroup analysis

No predefined subgroups analyses were planned or performed, which is acceptable for this type of study.

Drug-drug interactions

No drug-drug interaction studies have been conducted. This is acceptable considering that the safety related to drug interaction profile of the candidate biosimilar BP13 is expected to be same as that of the reference product Neupogen.

Discontinuations due to AEs

Only one person in the study discontinued due to AEs. This was a single mild, Grade 1 case of urticaria in the BP13 study group, treated with cetirizine, which subsequently resolved. No new safety findings were evident from this single case.

Long-term data

The duration of the study was 15 days. No longer term data are available from any of the participating subjects. In this setting, it is considered, as per guidance, acceptable.

BP13 has not been marketed to date, hence, no post marketing data are available for this product.

In conclusion, the safety profile of BP13 in a study population of healthy males (study BP13-101), for the duration of 15 days, was consistent and comparable to the safety profile of the originator Neupogen and did not show any new or unexpected safety signals. The observed safety profile of BP13 can be considered similar to the known safety profile of Neupogen.

2.5.8. Conclusions on the clinical safety

The safety profile of BP13 appeared consistent and comparable to the safety profile of the reference product Neupogen. No new or unexpected safety signals were identified. Thus, also with reference to current guidance (EMEA/CHMP/BMWP/31329/ 2005 Rev 1), the provided safety data support biosimilarity.

2.6. Risk Management Plan

2.6.1. Safety concerns

None.

2.6.2. Pharmacovigilance plan

No additional pharmacovigilance activities.

2.6.3. Risk minimisation measures

No additional risk minimisation measures.

2.6.4. Conclusion

The CHMP considers that the risk management plan version 0.1 is acceptable.

2.7. Pharmacovigilance

2.7.1. Pharmacovigilance system

The CHMP considered that the pharmacovigilance system summary submitted by the applicant fulfils the requirements of Article 8(3) of Directive 2001/83/EC.

2.7.2. Periodic Safety Update Reports submission requirements

The requirements for submission of periodic safety update reports for this medicinal product are set out in the list of Union reference dates (EURD list) provided for under Article 107c(7) of Directive

2001/83/EC and any subsequent updates published on the European medicines web-portal.

2.8. Product information

2.8.1. User consultation

The results of the user consultation with target patient groups on the package leaflet submitted by the applicant show that the package leaflet meets the criteria for readability as set out in the *Guideline on the readability of the label and package leaflet of medicinal products for human use.*

2.8.2. Additional monitoring

Pursuant to Article 23(1) of Regulation No (EU) 726/2004, Zefylti (filgrastim) is included in the additional monitoring list as it is a biological product.

Therefore, the summary of product characteristics and the package leaflet includes a statement that this medicinal product is subject to additional monitoring and that this will allow quick identification of new safety information. The statement is preceded by an inverted equilateral black triangle.

3. Biosimilarity assessment

3.1. Comparability exercise and indications claimed

Zefylti (BP13) was developed as a proposed biosimilar to the reference product Neupogen (filgrastim). The applicant is claiming all of the approved indications for Neupogen.

The proposed indications are:

Zefylti is indicated for the reduction in the duration of neutropenia and the incidence of febrile neutropenia in patients treated with established cytotoxic chemotherapy for malignancy (with the exception of chronic myeloid leukaemia and myelodysplastic syndromes) and for the reduction in the duration of neutropenia in patients undergoing myeloablative therapy followed by bone marrow transplantation considered to be at increased risk of prolonged severe neutropenia. The safety and efficacy of Zefylti are similar in adults and children receiving cytotoxic chemotherapy.

Zefylti is indicated for the mobilisation of peripheral blood progenitor cells (PBPCs).

In patients, children or adults, with severe congenital, cyclic, or idiopathic neutropenia with an ANC of ≤0.5 x 10^9/L, and a history of severe or recurrent infections, long term administration of Zefylti is indicated to increase neutrophil counts and to reduce the incidence and duration of infection-related events.

Zefylti is indicated for the treatment of persistent neutropenia (ANC less than or equal to 1.0 x 10^9/L) in patients with advanced HIV infection, in order to reduce the risk of bacterial infections when other options to manage neutropenia are inappropriate.

Summary of quality comparability data

The applicant has performed comprehensive analytical testing of batches of Zefylti FP and batches of EU-Neupogen. FP batches were sourced from different AS batches. FP batches include clinical batches, process validation batches, and the proposed commercial representative batches.

Overall, the proposed biosimilarity approach follows the general principles outlined in the guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance. Relevant physicochemical, structural, and functional attributes were compared with a panel of state-of-the-art and standard methods. In addition to analytical assessment, comparative forced degradation studies were performed. According to the applicant, all the assays used in the biosimilarity are suitable for their intended purpose. Batch release analytical methods were validated, and others qualified.

Based on the provided data, Zefylti could be considered as a biosimilar to EU-Neupogen.

Summary of non-clinical comparability data

The primary pharmacodynamic studies included *in vitro* comparison of primary functions of filgrastim, *i.e.* binding to G-CSFR and initiating cell proliferation. In addition, similarity of immunogenicity of BP13 and Neupogen was assessed in three *in vitro* analyses. The non-clinical biosimilarity data package does not contain any pharmacokinetic or *in vivo* toxicity studies.

Summary of clinical comparability data

One PK/PD study (BP13-101) was conducted. This was a multiple-dose (5µg/kg/day SC from day 1 to day 5), randomised, double-blind, parallel study in healthy adult male subjects comparing BP13 and Neupogen (N =74 randomised subjects in BP13 group and N =72 randomised subjects in Neupogen group). Safety and immunogenicity were assessed as secondary endpoints of this study.

The PK/PD study was performed in accordance with the guideline on similar biological medicinal products containing G-CFS (EMEA/CHMP/BMWP/31329/2005 Rev1).

3.2. Results supporting biosimilarity

Quality data

Most of the quality attributes proved to be highly similar. In cases where certain data points were at the edge / slightly out of the pre-established similarity criteria a sufficient justification that these results do not have any impact on the efficacy and safety profile of filgrastim was provided. In addition, based on the comparative force degradation studies, the degradation pathway, degradation products, and degradation rates of Zefylti and EU approved Neupogen were found to be comparable.

Results supported similarity for the following properties:

- Protein content
- Primary structure
- Size heterogeneity
- Charge heterogeneity
- Structural heterogeneity
- Post-translational modifications
- Higher order structure
- Functional properties
- Lack of activation of Toll-like receptors
- Deliverable/extractable volume, osmolality, pH and excipients content
- Stability under accelerated and stressed conditions and forced degradation

Non-clinical data

The *in vitro* data support the similarity of BP13 and Neupogen in binding to G-CSFR, activation of phospho-STAT3 signalling downstream receptor binding and initiating cell proliferation, and lack of

activation of Toll-like receptors and in triggering the cytokine secretion and T-cell proliferation (immunogenicity characteristics).

Clinical data

Pharmacokinetics

In the comparison of PK data between BP13 and Neupogen (study BP13-101), the 90%CIs of the geometric LS mean ratios for two primary PK parameters (i.e., C_{max} and $AUC_{(0-t)}$), were within the acceptance range of 80-125% (including 100). The secondary PK parameters were at similar levels between the test and the reference product group.

Pharmacodynamics

The pharmacodynamics of BP13 was investigated as part of study BP13-101. In terms of the primary PD endpoints, the geometric mean ratio (95% CI) was 0.990 (0.904, 1.084) for ANC AUEC(0-t) and 1.002 (0.926, 1.084) for ANC Emax, i.e., the 95% CIs were within the acceptance range of 90-111%. The secondary endpoints $CD34^+$ AUEC (2-t), $CD34^+$ Emax and T max for ANC and $CD34^+$ supported the overall conclusion of biosimilarity in terms of PD.

Safety and Immunogenicity

Safety and immunogenicity were investigated as a secondary objective of the study BP13-101 in healthy volunteers. Overall, the provided safety database can, as per guidance, be considered sufficient for the establishment of similar safety profile between BP13 and Neupogen. This is also considering the well-known safety profile of this active substance and its nature, i.e., a biosimilar.

A total of 344 subjects were screened for the study BP13-101 and 146 were randomised, with 74 subjects randomised to BP13 and 72 subjects to Neupogen. Overall, 144/146 (98.6%) of the randomised subjects were included in Safety Analysis Set. In all, 135/146 (92.5%) subjects completed the 15-day study, 90.5% in the BP13 arm and 94.4% in the Neupogen arm.

The most commonly reported PTs were headache, back pain, bone pain and catheter site pain. All other TEAEs were mainly single occurrences. No major imbalances were identified between the treatment groups. A total of 46 events in 42/144 (29.2%) subjects were considered to be probably related to study drug (25 events in 22/72 [30.6%] subjects in the BP13 arm and 21 events in 20/72 [27.8%] subjects in the Neupogen arm) and a total of 125 events in 87/144 (60.4%) subjects were considered to be possibly related to study drug (66 events in 46/72 [63.9%] subjects in the BP13 arm and 59 events in 41/72 [56.9%] subjects in the Neupogen arm). No deaths or SAEs were reported in either treatment arm. According to the applicant, no relevant trends were identified in the investigated clinical laboratory parameters, vital signs, or ECG results and none of the abnormal results reported for these evaluations were considered clinically significant. Moreover, none of the subjects in BP13 arm and Neupogen arm were confirmed to be ADA positive at any time-point of the study. Overall, no new or unexpected safety finding were evident.

The safety profile of the candidate biosimilar BP13, in the study population of 144 healthy males (study BP13-101), for the duration of 15 days, appeared consistent and comparable to that of the safety profile of the reference product Neupogen.

3.3. Uncertainties and limitations about biosimilarity

There are no remaining uncertainties and limitations that have an impact on the conclusion of biosimilarity.

3.4. Discussion on biosimilarity

Quality

Overall, the proposed biosimilarity approach follows the general principles as outlined in the guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance. Based on the provided data it is agreed that similarity is demonstrated and Zefylti is considered as a biosimilar to EU-Neupogen.

Non-clinical

The comparative *in vitro* data package appears somewhat limited to demonstrate the similar functional activity of BP13 and Neupogen. Nevertheless, these studies reflect the principal mode of action of filgrastim, i.e., binding to G-CSFR (on bone marrow precursor cells) followed by cell proliferation.

In addition to principal pharmacodynamic activity analyses, three immunogenicity/immunotoxicity assays were conducted. In these assays, lack of Toll-like receptor activation was demonstrated in BP13 and Neupogen. In addition, based on the provided further clarification on questions raised at D80 assessment, the potential for T-cell mediated immunogenicity and triggering the cytokine secretion in PBMCs was similar for BP13 and Neupogen.

Clinical

Biosimilarity in the PK/PD study BP13-101 using healthy male adult subjects was formally demonstrated between BP13 and Neupogen as in the primary PK parameters (i.e., C_{max} and $AUC_{(0-t)}$), the 90% CI for the ratio of test-to-reference fell within the acceptance range of 80-125%.

In terms of the primary PD endpoints ANC AUEC(0-t) and ANC Emax, the geometric mean ratios (95% CI) were within the acceptance range of 90-111% and the secondary endpoints supported the overall conclusion of biosimilarity in terms of PD.

In terms of safety and immunogenicity, based on the provided data, the safety profile of BP13 is considered overall to be similar to that of reference medical product Neupogen.

Multidisciplinary immunogenicity summary

Regarding immunogenicity, the applicant was requested to justify that that there is no unwanted immune response compared to Neupogen. Importantly, from the quality point of view, the quality attributes potentially contributing to increase in immunogenicity, including protein aggregation, were demonstrated to be highly similar. However, in non-clinical assays there were differences in BP13 batches in terms of immunogenicity. One batch (R1302-CL-0001) out of the three tested was systematically triggering more secretion of cytokines in PBMC and had a higher T-cell-mediated immunogenicity risk as suggested in the T-cell proliferation assay, and this was further asked to be clarified by the applicant during D80 assessment. Based on the information obtained, it was concluded that BP13 and Neupogen could be considered similar in their *in vitro* potential to trigger cytokine secretion and T-cell proliferation within the study conditions.

Further, in the clinical study none of the subjects in BP13 arm and Neupogen arm were confirmed to be ADA positive at any time-point of the study. It is acknowledged that antibodies against rG-CSF have been reported to develop infrequently and have previously not been associated with relevant consequences for efficacy or safety. In spite of this, the complete lack of ADA response was somewhat unexpected. Thus, the applicant, overall clarified this issue, but no apparent reason for this finding was clearly evident.

3.5. Extrapolation of safety and efficacy

The applicant is claiming all indications for the reference product Neupogen. According to the draft guideline (EMEA/CHMP/BMWP/31329/2005 Rev 1), pivotal evidence for similar efficacy can be derived from the similarity demonstrated in physicochemical, functional, PK and PD comparisons, and therefore a dedicated comparative efficacy trial is not considered necessary. Furthermore, considering that G-CSF has only a single mode of action, i.e., through binding to the G-CSF receptor, it can be agreed that all indications of Neupogen can be also approved for BP13.

3.6. Additional considerations

Not applicable.

3.7. Conclusions on biosimilarity and benefit risk balance

Based on the review of the submitted data, Zefylti is considered biosimilar to Neupogen. Post authorisation measures (REC) have been agreed.

A benefit/risk balance comparable to the reference product can be concluded.

4. Recommendations

Outcome

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considers by consensus that the benefit-risk balance of Zefylti is favourable in the following indication(s):

Zefylti is indicated for the reduction in the duration of neutropenia and the incidence of febrile neutropenia in patients treated with established cytotoxic chemotherapy for malignancy (with the exception of chronic myeloid leukaemia and myelodysplastic syndromes) and for the reduction in the duration of neutropenia in patients undergoing myeloablative therapy followed by bone marrow transplantation considered to be at increased risk of prolonged severe neutropenia.

The safety and efficacy of Zefylti are similar in adults and children receiving cytotoxic chemotherapy.

Zefylti is indicated for the mobilisation of peripheral blood progenitor cells (PBPCs).

In patients, children or adults, with severe congenital, cyclic, or idiopathic neutropenia with an absolute neutrophil count (ANC) of ≤ 0.5 x 10^9/L, and a history of severe or recurrent infections, long term administration of Zefylti is indicated to increase neutrophil counts and to reduce the incidence and duration of infection-related events.

Zefylti is indicated for the treatment of persistent neutropenia (ANC less than or equal to 1.0 x 10^9/L) in patients with advanced HIV infection, in order to reduce the risk of bacterial infections when other options to manage neutropenia are inappropriate.

The CHMP therefore recommends the granting of the marketing authorisation subject to the following conditions:

Conditions or restrictions regarding supply and use

Medicinal product subject to restricted medical prescription (see Annex I: Summary of Product Characteristics, section 4.2).

Other conditions and requirements of the marketing authorisation

- **Periodic Safety Update Reports**

The requirements for submission of periodic safety update reports for this medicinal product are set out in the list of Union reference dates (EURD list) provided for under Article 107c(7) of Directive 2001/83/EC and any subsequent updates published on the European medicines web-portal.

Conditions or restrictions with regard to the safe and effective use of the medicinal product

- **Risk Management Plan (RMP)**

The marketing authorisation holder (MAH) shall perform the required pharmacovigilance activities and interventions detailed in the agreed RMP presented in Module 1.8.2 of the marketing authorisation and any agreed subsequent updates of the RMP.

An updated RMP should be submitted:

- At the request of the European Medicines Agency;
- Whenever the risk management system is modified, especially as the result of new information being received that may lead to a significant change to the benefit/risk profile or as the result of an important (pharmacovigilance or risk minimisation) milestone being reached.

Conditions or restrictions with regard to the safe and effective use of the medicinal product to be implemented by the Member States

Not applicable.